Synthesis of silicon functionalised cyclic peptides for enantiomeric separations

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SYNTHESIS OF SILICON FUNCTIONALISED CYCLIC PEPTIDES FOR ENANTIOMERIC SEPARATIONS.

submitted by Kim Kai Wai Wong

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ABSTRACT.

The work described in this thesis has been divided into three sections, INTRODUCTION (section one), RESULTS AND DISCUSSION (section two) and EXPERIMENTAL (section three).

Section one, CHAPTER ONE, gives a brief discussion of the methods of peptide synthesis that have been used for the synthesis of the target compounds described in the EXPERIMENTAL section. In addition, solid phase peptide synthesis is also briefly discussed, as are the recent developments in peptide bond formation. The coverage is not comprehensive and the reader is referred to the appropriate references as indicated in the text.

Subsequent chapters, CHAPTERS TWO-SIX, in section one discuss the various methods that have been used for enantiomeric separation, their mechanism and application. The chapters are broadly divided between the two methods, indirect and direct method, the latter describing the various modes that can be used to achieved such separations. The coverage is more comprehensive in order to give a detailed insight into this increasingly important area of analytical chemistry. In addition, computer assisted molecular modelling is discussed briefly.

Section two, CHAPTER SEVEN describes the convergent routes that were used for the synthesis of the cyclic peptides, and also an alternative for the synthesis of

unsaturated glycine derivatives. CHAPTER EIGHT and NINE describes the synthesis of the cyclic peptides and their functionalisation to silicon centres briefly, the mechanism of reaction, their identification and characterisation using a variety of methods. Modification of silica gel with the silicon functionalised cyclic peptides is also described. CHAPTER TEN describes the evaluation of the modified silica gel, described in CHAPTER NINE, using high performance liquid chromatography. Chromatograms of the actual separations observed are included.

Section three, CHAPTER ELEVEN describes in detail the synthetic procedure for each compound, together with characterisation data for that compound.

ACKNOWLEDGEMENTS.

The research project described in this thesis was carried out between October 1986 and September 1989 in the Department of Organic Chemistry at the University of Bath.

I am indebted to Dr. Brian Brisdon and Dr. David Brown for their help, advice, encouragement, humour and endless patience in both the practical work, and the writing of this thesis.

My thanks also go to Dr. Dick Kinsman and Dr. Peter White for their advice and help in the preliminary stages of this project. I would also like to thank my colleges in the Organic Chemistry Department for their advice, friendship and making my stay at Bath an enjoyable one. I would particularly like to express many heartfelt thanks to my dear friends, Colin Williams, Debbie Foster, Dr. Jennifer Bennett and the staff of the Medical Centre with whom I have had the pleasure of sharing many memorable times. Thanks are also extended to Sue, Richard, John and Russel of the Organic Chemistry Department for their technical assistance, Chris for mass spectroscopy services, Alan for elemental analyses, Mary for X-ray crystallographic services and to Dave and Harry for nuclear magnetic resonance spectroscopy services.

Financial support from the Science and Engineering Research Council for this project is gratefully acknowledged.

In Memory Of Diane Jackson.

A Lost Friend.

Died 21 April 1988.

Sanity is but a figment of the imagination.

Kim Kai Wai Wong

CONTENTS.

ABBREVIATIONS

$(Boc)_2O$	di-tert-butyloxycarbonyl anhydride
2,2 DMP	2,2-dimethoxypropane
AcOH	acetic acid
α_1-AGP	α_1-acid-glycoprotein
Ar	aromatic
asym	asymmetric
b	broad
BSA	bovine serum albumin
CAMM	computer assisted molecular modelling
CDA	chiral derivatising agent
CMP	chiral mobile phase
CMPA	chiral mobile phase additive
CSA	chiral solvating agent
CSP	chiral stationary phase
d	doublet
DCCI	dicyclohexylcarbodimide
DCM	dichloromethane
DCU	dicyclohexylurea
dec	decomposes
def	deformation
DMF	*N*,*N*-dimethylforamide
ether	diethyl ether
EtOAc	ethylacetate
GC	gas chromatography
Gly	glycine

h	heptet
HOBt	1-hydroxybenzotriazole
HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
IBC	isobutylchloroformate
IR	infrared spectroscopy
$KOBU^t$	potassium-tert-butoxide
LC	liquid chromatography
LEC	ligand exchange chromatography
Leu	leucine
LUMO	lowest unoccupied molecular orbital
m	multiplet
MeOH	methanol
$MgSO_4$	anhydrous magnesium sulphate
MS	mass spectroscopy
NBS	*N*-bromosuccinimide
NMM	*N*-methylmorpholine
NMR	nuclear magntic resonace spectroscopy
pet ether	ether petroleum ether 60-80^{0}C
pNP	para-nitrophenol
q	quartet
quin	quintet
R.T.	room temperature
R_t	retention time
s	singlet
sex	sextet
str	stretch
sub	sublimes

sym	symmetric
t	triplet
TAC	cellulose triacetate
TAPA	α-(2,4,5,7-tetranitro-9-flurenylidene-aminooxy)-propionic acid
TEA	triethylamine
TFA	trifluoroacetic acid
UV	ultra violet
X-ray	X-ray crystallography

INTRODUCTION

CHAPTER ONE.

Introduction.

For many reasons there is an ever increasing need to separate racemic mixtures into their components, and there has in recent years been an explosive increase in the use of high performance liquid chromatography (HPLC) and chiral stationary phases (CSP) to perform such tasks, especially in the fine chemicals and pharmaceuticals industries.

In 1970, Losse *et al*[1] described the state of chromatographic resolutions of racemates as follows:- "The survey shows that reliably reproducible separation effects can now be demonstrated on many asymmetric carriers and phases."

From 1975-1990 the use of many different CSPs for enantiomeric separation was recorded in the literature.[2,3] Many, however, require that the analyte be derivatised in order to achieve or enhance separation of the enantiomers, and many recent articles have updated the recent developments that have sought to overcome this drawback to the method.[3-7]

Alternatively in order to avoid the separation problem entirely, an asymmetric synthesis, in which prochiral groupings are converted to chiral groups, can be employed in order to prepare a specific enantiomer. Practical asymmetric syntheses are of two types. The majority involve addition to some unsaturated grouping and the selection of

enantiotopic or diastereotopic faces, to give a single enantiomer or a pair of diastereomers. Less common, is the selective substitution or modification of paired ligands at a prochiral centre.[8-10] In 1974 Eliel[11] established three guidelines for an effective asymmetric synthesis:-

a) it must lead to the desired enantiomer in high optical, as well as chemical, yield;

b) the chiral product must be readily separable from the chiral auxiliary reagent needed in the synthesis;

c) unless the chiral reagent is very much less expensive than the desired product, it must be possible to recover the auxiliary reagent in good yield and in undiminished purity.

If the chiral reagent is a catalyst capable of functioning efficiently at a low catalyst/substrate ratio, ie turnover is high, recovery of the reagent might be unnecessary.

Although many asymmetric syntheses are being achieved, practical difficulties, and economic considerations are likely to ensure that even on a preparative scale, the use of direct HPLC methods for the resolution of racemic mixtures will continue unabated. But, in particular the pressure on developmental chemists in the fine chemical and pharmaceutical industries, to demonstrate the absence of a biologically undesirable enantiomer in an optically active product, will ensure improvements in the analytical applications of the technique. The consequences of failure in this area can be severe as was unforgettably demonstrated by the thalidomide

tragedy.[12]

The aim of this project was to synthesise small functionalised cyclic peptides, which could be chemically bonded to a variety of silicon centres, including silica and siloxanes, so permitting their evaluation for enantiomeric separations. The advantage of such an approach lies in the fact that it is entirely feasible to "construct" a cyclic peptide that is stereoselective when used in such a manner. If necessary, the "construction" can be performed using computer assisted molecular modelling techniques (CAMM) to generate possible host-guest docking shapes on the basis of preferred conformation and optimisation of hydrogen bonding and other electrostatic forces between the peptide and a specific guest enantiomer. Appropriate modification of the cyclic peptide can then be made, after experimental assessment, with a view to improving the binding. The molecular modelling, peptide modification, selective retention cycle should allow optimisation for a given host-guest complex and in the longer term, should lead to predictions concerning suitable hosts for specific substrate types.

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CHAPTER TWO.

PEPTIDE SYNTHESIS.

2.1 Introduction.

This chapter contains a summary of the important methods used for peptide bond formation in solution. The coverage cannot be comprehensive and more specific details are available in the texts noted in reference 1. In addition solid phase peptide synthesis is dealt with briefly. The methods that will be considered are:-

2.2.1 The acid chloride method

2.2.2 The acid azide method

2.2.3 Anhydride method

2.2.4 Active ester method

2.2.5 Coupling reagents

2.2.6 Enzyme catalysed bond formation

2.3 Solid phase peptide synthesis

In order to promote the formation of an amide bond, it is necessary to activate the carbon atom of the carbonyl group with an electron withdrawing substituent, X, so that its electrophilicity is enhanced towards nucleophilic attack by the amino group (Fig 2.1). This may be achieved in several ways as described in the following sections.

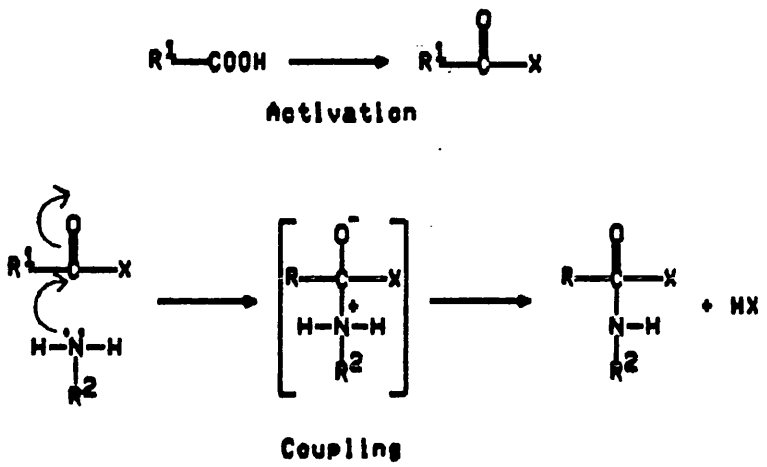

Fig 2.1

2.2 Peptide Bond Formation In Solution.

2.2.1 The acid chloride method.

The chlorine atom is an obvious and well established choice for activating the carbon atom of the carbonyl group. Yet amino acid chlorides were used only infrequently for peptide synthesis in the early part of this century.[2] Initially, the carbonyl group of the protected amino acid was converted to the chloride by treatment at room temperature with phosphorus pentachloride (Fig 2.2). However, the phosphorus oxychloride so formed is

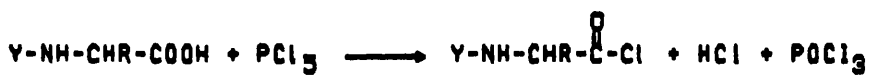

Fig 2.2

difficult to remove from the product, and consequently thionyl chloride was soon preferred (Fig 2.3). Once formed,

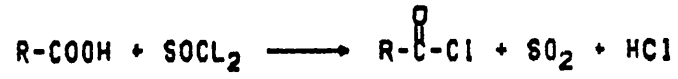

Fig 2.3

these acid chlorides may be subject to intramolecular attack on the acid chloride group by a weak, but favourably placed nucleophile within the carbonyl component. This occurs in alkoxycarbonyl blocking groups, such as benzyloxycarbonyl amino acid chlorides (Fig 2.4),

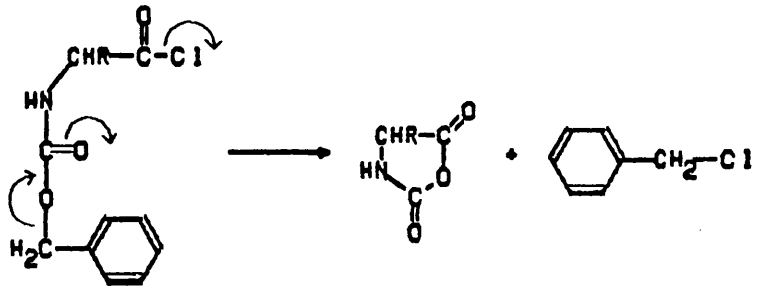

Fig 2.4

giving rise to *N*-carboxyanhydrides (Leuchs anhydrides).[3] Although these derivatives are suitable for acylations, they cannot be used when a well defined sequence is required. Intramolecular nucleophilic attack in acyl amino acid chlorides, yielding oxazolones/azalactones,[4] can also occur, and are often implicated in racemisation (Fig 2.5). Attempts to revive the acid chloride method using, for

example oxalyl chloride[5] or *N,N*-dimethylformamidium chloride[6] (from dimethylformamide and phosgene) have met with little success.

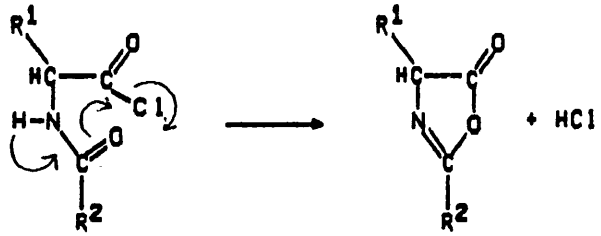

Fig 2.5

2.2.2 The acid azide method.

The only contemporary, viable alternative to the acid chloride method was the acid azide method originated by Curtius.[7] It remains a widely used method, the chief attraction being the resistance of the azide activated peptide derivatives to racemisation. The azides are formed from hydrazides, which in turn are formed from hydrazinolysis of alkyl esters, using nitrous acid (Fig 2.6). In a useful modification of the Curtius method hydrazides are converted to azides using alkyl nitrite,[8] under anhydrous conditions, thus avoiding some side reactions. A further improvement was introduced by Shiori

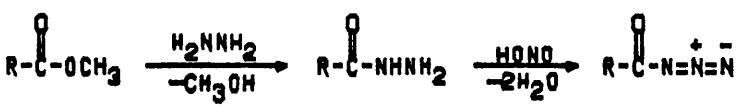

Fig 2.6

et al,[9] whereby a free carbonyl group can be directly converted to the azide using diphenylphosphorylazide (Fig 2.7). However, none of these modifications circumvent the main shortcoming of the azide method, which involves the rearrangement of the azide to an isocyanate, the Curtius

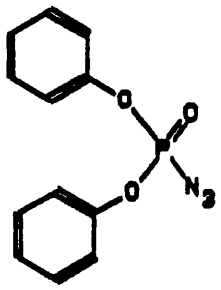

Fig 2.7

rearrangement[10] (Fig 2.8). The isocyanates can react with the amine component to produce urea derivatives (Fig 2.9), which, since they resemble the desired peptide, can only be

Fig 2.8

Fig 2.9

removed with considerable difficulty. This side reaction can be minimised if low temperatures and high concentrations of reactants are used.

2.2.3 Anhydride method.

Perhaps the simplest and most efficient method of acylation is to treat amines (or other nucleophiles) with carboxylic anhydrides. However, the anhydride method remained unexploited for several decades, the chief reason being the wastefulness of the method (Fig 2.10). However, more recently, protected amino acids are being prepared with increasing facility and decreasing cost, and their anhydrides are more often used. An infrequently used,

Fig 2.10

but novel approach to the synthesis of symmetrical anhydrides involves reaction of the acid with phosgene[11] (Fig 2.11).

Unsymmetrical/mixed anhydrides are more widely used in peptide synthesis and the intermediates are constructed such that the blocked amino acid is the sole acylating moiety, while the acid playing the role of "activator" is eliminated. Originally acetic acid and benzoic acid were used as "activators", but the electrophilic character of the two carbonyl groups was not sufficiently pronounced to

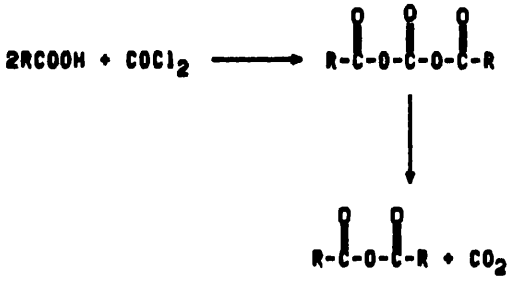

Fig 2.11

ensure unambiguous nucleophilic attack from the amino component. Vaughan and Osato[12] introduced the use of long chain fatty acids, such as isovaleric acid, to act as the activator, so that the "activating" carbonyl function becomes both electronically and sterically less electrophilic, (Fig 2.12). Nucleophilic attack by the amine component will therefore occur mainly at the carbonyl

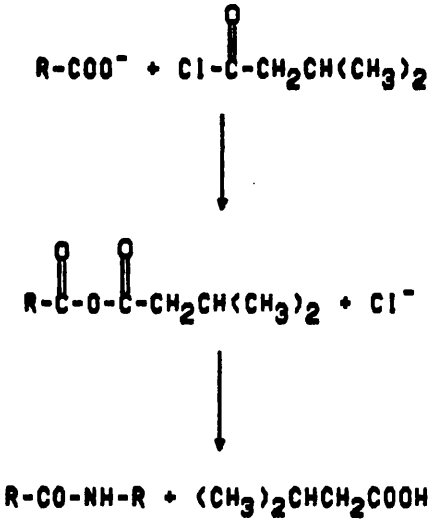

Fig 2.12

group of the protected amino acid, and usually the second acylation product, an isovalerylamide is produced in very small, often negligible, amounts. An enhancement of this steric-electronic effect can be achieved using trimethylacetic acid (pivalic acid) mixed anhydrides.[13]

The introduction of alkyl chlorocarbonates or chloroformates marked an important development of the mixed anhydride method (Fig 2.13). The use first of ethylcarbonic acid mixed anhydrides,[14,15] and latterly, isobutylcarbonic acid mixed anhydrides,[16,17] quickly gained wide acceptance in

Fig 2.13

peptide synthesis, as the products of acylation are carbon dioxide and an alcohol, both of with can be easily removed from the product (Fig 2.14). In general,this procedure produces a small amount of the second acylation product, a

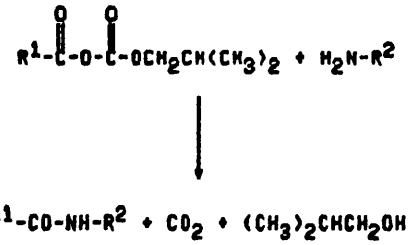

Fig 2.14

urethane, a complication not possible with symmetrical anhydrides, but the advantages of the method have led to its wide acceptance.

2.2.4 Active ester method.

By using active esters the ambiguity of two carbonyl groups, via coupling mixed anhydrides is avoided, as there is only one electrophilic centre available for acylation. Simple alkyl esters have only modest reactivity. However their reactivity is increased by C-substitution with strongly electron withdrawing groups, such as the cyano-group. Activated alkyl esters have been used for peptide synthesis,[18,19] but satisfactory rates of reaction were only observed when the reactants were in a high concentration, so limiting the synthesis to dipeptides and short chain peptides. The use of aryl esters broadened the applicability of the method, as demonstrated by Bodansky,[20] in reactions involving p-nitrophenyl esters and o-nitrophenyl esters. The latter, which is highly activated shows little dependence on the nature of the solvent. The former is most active in highly polar media, such as DMF and DMSO. Despite this solvent dependency, the para-derivatives are widely used, due mainly to the ease with which the derivatives crystallise. In addition, the introduction of the catalyst, 1-hydroxybenzotriazle (HOBt),[21] reduced the differences in reactivity between the two reagents, due to the formation of a ternary complex between the active ester and the catalyst (Fig 2.15).

Esters of HOBt are very potent acylating agents, probably due to anchimeric assistance[22, 23] (Fig 2.16). Similar catalytic affects are observed with other *N*-hydroxy compounds, such as *N*-hydroxypiperidine.[24]

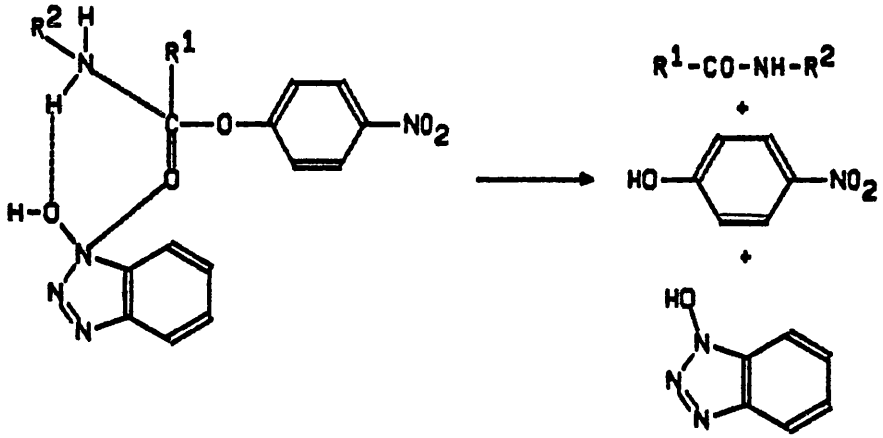

Fig 2.15

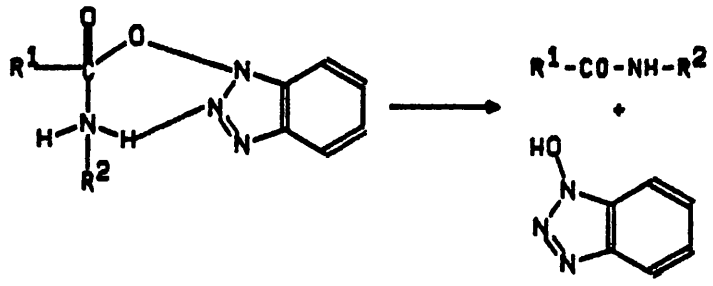

Fig 2.16

The developing interest in the synthesis of biologically active peptides, such as oxytocin, stimulated studies on esters of even higher activity. Thus *N*-hydroxysuccinimide[25] has proved popular for use in peptide synthesis (Fig 2.17), but its use has drawbacks[23] as the succinimide carbonyl group is susceptible to nucleophilic

attack. Nevertheless, the side product, N-hydroxysuccinimide, can be readily removed by extraction with water, and the reagent remains an attractive one.

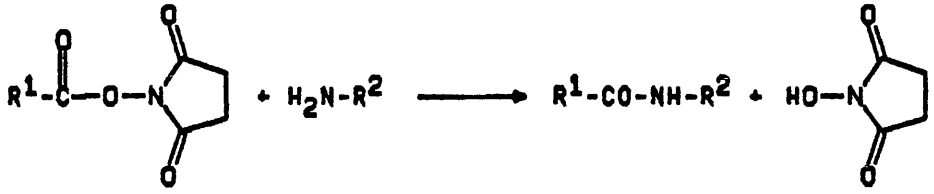

Fig 2.17

For the preparation of amino acid polymers, pentachlorophenyl esters are recommended.[27] However, the bulkiness of the activating group may outweigh the high degree of activation it provides when steric hinderance is a binding factor as in some solid phase peptide syntheses. This steric problem may be overcome somewhat by using either pentafluorophenyl esters[28] or 2,4,5-trichlorophenyl esters[29] (Fig 2.18).

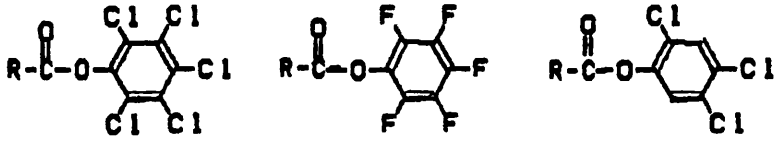

Fig 2.18

2.2.5 Coupling reagents.

The criteria for coupling reagents are very

stringent:-

1) it should be inert toward the amine component (already present during activation),

2) it should not generate a reactive intermediate that possess a nucleophilic centre (as this can compete with the amine component for the acyl group),

3) it should not cause overactivation which would lead to side reactions, and hence by-products.

A great advance in the history of peptide bond formation occurred on the introduction of the carbodimide coupling reagents,[30] of which dicyclohexylcarbodimide (DCCI) is especially noteworthy. The coupling reagents can be added directly to the reaction mixture of the carbonyl and amine component, thus enabling activation and coupling to proceed simultaneously. Although amines react with carbodimides to give guanidine derivatives,[28] this reaction is too slow to compete significantly with the rapid addition of the carbonyl group (Fig 2.19). The N=C group, found in the O-acylisoureas formed from the addition of the carboxylic acid to the carbodimide, is powerfully activated, leading to facile coupling (Fig 2.20).

The use of DCCI has become very popular as it promotes a rapid rate of reaction, and allows coupling to be achieved in a single operation. However, it has the disadvantage of generating the by-product dicyclohexylurea (DCU), which has a low, but significant solubility in most organic solvents typically used for the reaction. Hence this impurity cannot be removed entirely by filtration, and

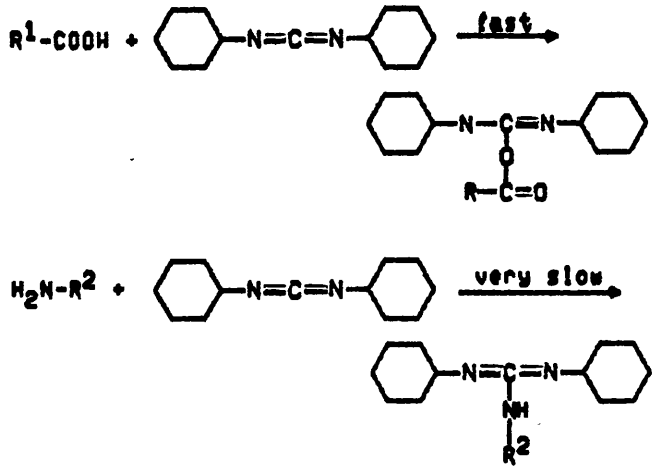

Fig 2.19

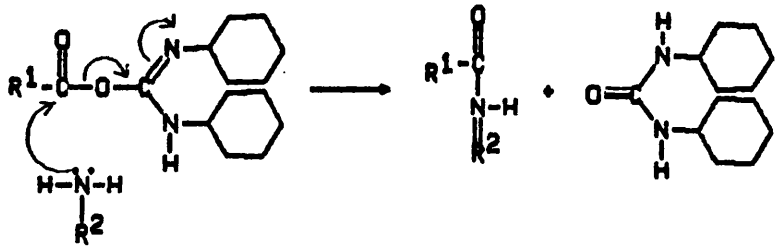

Fig 2.20

remains a contaminant in the product. The introduction of water soluble carbodimides[32, 33] overcomes this problem.

Another coupling reagent, carbonyldiimidazole,[34] mediates coupling via reactive N-acyl intermediates (Fig 2.21). Unfortunately the reagent is very sensitive to moisture, and is fairly expensive to prepare. The more convenient reagents, 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ),[35] and the improved version 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ),[36] readily generate alkyloxycarbonic acid mixed

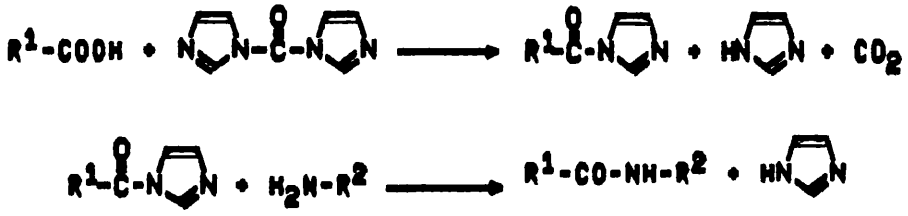

Fig 2.21

anhydrides, offering some advantages in the coupling process (Fig 2.22). An important feature of EEDQ and IIDQ mediated couplings is the reasonable conservation

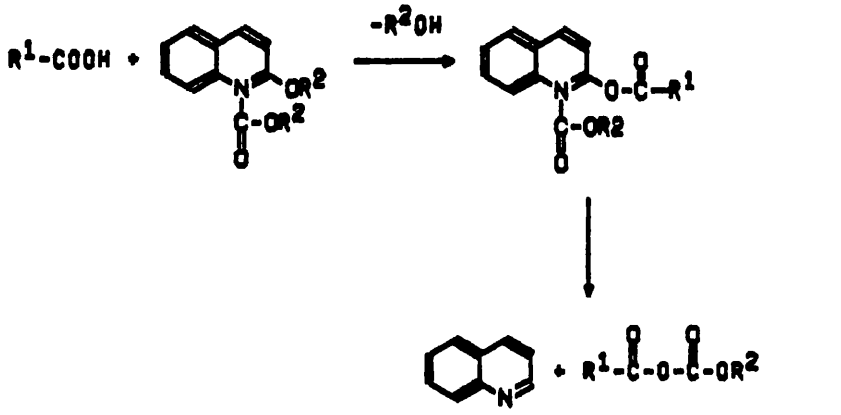

Fig 2.22

of chiral purity. This can be attributed to the lack of proton abstraction in EEDQ and IIDQ, and in the reactive intermediates. Since quinoline is released during activation, no additional base is needed to neutralise the HCl formed when mixed anhydrides are prepared via chlorocarbonates, and the base is not strong enough to cause racemisation by proton abstraction.

Less successful alternatives to DCCI are Bates reagents (Fig 2.23),[37] and the benzotriazolyl-N oxytri-dimethylamino-phosphonium hexafluorophosphate (BOP) reagent (Fig 2.24).[38]

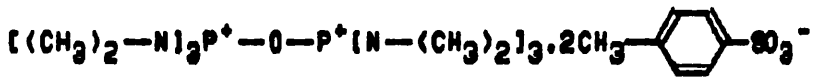

Fig 2.23

It is often less demanding, to separate the activation and coupling steps, eg., DCCI often gives better results when used in activating mode, and the symmetrical anhydrides formed applied for the required reaction to the amino component.

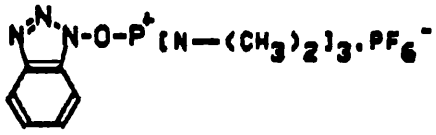

Fig 2.24

2.2.6 Enzyme catalysed bond formation.

Peptide bonds are quite resistant to hydrolysis in the absence of acid or base, but this reaction is accelerated greatly by the presence of proteolytic enzymes. This has prompted attempts to use enzymes for the synthesis of, rather than the hydrolysis of, peptide bonds.[39,40] As enzymes act only as catalysts, so accelerating the establishment of

equilibria, proteolytic enzymes can be used for amide bond formation, provided the equilibrium of the reaction is appropriate. Thus using papain, a vegetable pepsin, anilides of blocked amino acids can be prepared (Fig 2.25).

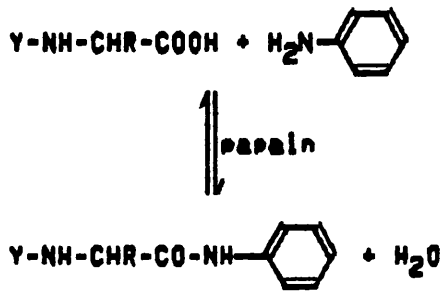

Fig 2.25

The anilide, being insoluble in water, precipitates, and provides the necessary driving force to shift the equilibrium toward synthesis. As the enzyme is specific for L-amino acids, the D-isomer in the starting material remains unchanged.

General applicability became more feasible on the introduction of water miscible organic solvents, such as glycerol and acetonitrile,[41] which affected the dissociation constants of the carboxyl group, in a selected pH region, and shifted the equilibria towards synthesis. An example is the conversion of porcine insulin to human insulin (Fig 2.26).[42] Removal of aniline from the C-terminus of the B-chain by trypsin is fairly selective. The desalanino pork insulin is produced in good yield, and then subjected to the action of trypsin with a large excess of L-threonine-tert-butyl ester. Acidolytic removal of the

tert-butyl ester affords the human insulin.

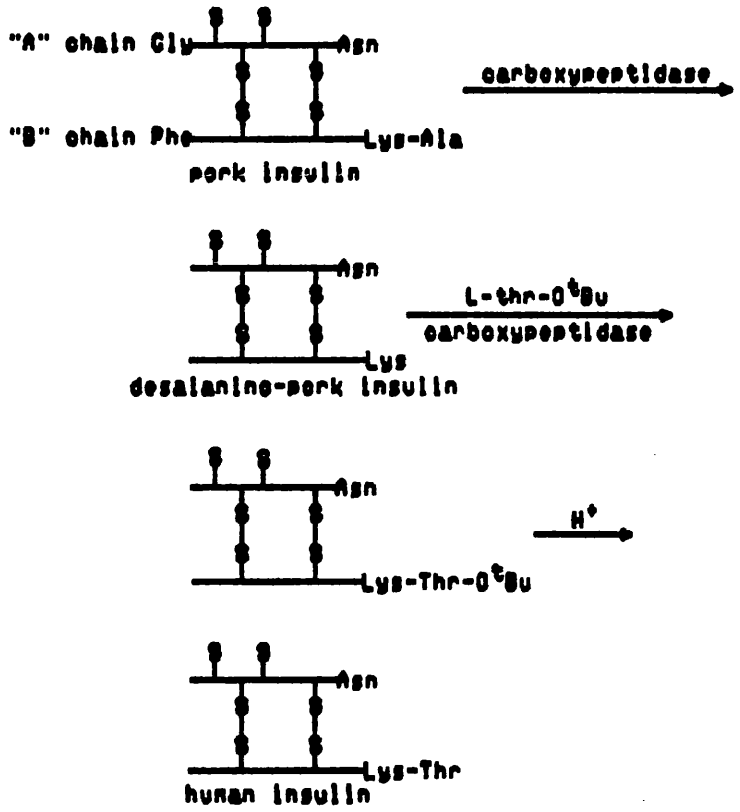

Fig 2.26

There have been some successful attempts to apply enzymes to the systematic building of peptide chains, such as the artificial sweetener, aspartame,[43] which could be prepared without blocking the β-carbonyl group of aspartic acid.

In general, enzymes lead to a great variation in coupling rates in reactions, which involve different amino acids, and so for the synthesis of various peptide bonds, individual optimum condition, and perhaps even the enzyme, must be carefully selected. Care must also be taken, under which conditions, the new peptide bond is formed due to the

possibility of hydrolysis of the other peptide bonds formed previously. It would appear that enzyme catalysed is synthesis will play a major role in the preparation of certain selected target compounds, but it is unlikely to replace conventional methods of synthesis in general peptide chemistry.

2.2.7 A comparison of the coupling methods.

The moderate stability of the reactive intermediate of the azide (sec 2.2.2), and of the symmetrical and mixed anhydrides (sec 2.2.3) is a common feature of both these methods. The intermediates, which can be isolated, cannot be stored for long periods of time. Azides slowly undergo Curtius re-arrangement, and mixed anhydrides disproportionate to symmetrical anhydrides, which even then have limited shelf life. Active esters (sec 2.2.4) are different in this respect, in that they can be isolated, and stored in a refrigerator, but this stability is offset by only moderate reactivity.

Hence the use of coupling reagents (sec 2.2.5) is frequently preferred, due to simplicity of use. There are some 40 different coupling reagents so far proposed in the literature, and yet most of them generate well known intermediates such as anhydrides or active esters. There is no doubt a bias towards one pot synthesis, but there is a price to be paid for such simplification in terms of purity, yield, and loss of chiral activity.

The use of active esters[44] as reactive intermediates

has advantages as the activation and coupling stages are separate, and the relative stability of protected, and activated amino acids allows them to be scrutinised before coupling. Bodansky advocated the use of active esters[44] in which the peptide chain is lengthened in a stepwise fashion. The condensation of peptide segments is perhaps best when coupling reagents are used, DCCI being the foremost among them, especially when used with axillary nucleophiles, such as HOBt.[22,23] Good results can also be achieved in segment condensations with EEDQ and IIDQ.[35,36] The use of proteolytic enzymes (sec 2.2.6) to catalyse syntheses of peptides holds great promise for the future.

2.2.8 Recent developments in peptide bond formation.

Complexes of amino acid chlorides with Pt(II) and Pd(II) have been prepared by Steiner *et al.*[44] Treatment of the corresponding amino acid complexes with a trialkylsilyl chloride gives $Cl_2M(NH_2.CHR'.CO_2SiR_3)_2$ which can be converted to $Cl_2M(NH_2.CHR'.COCl)_2$, by reaction with $COCl_2$. These can then be use to acylate amino acid esters and afford metal complexes of dipeptides esters.

Reactive esters derived from 2-chloro-4,6-dimethoxy-1,3,5-triazine have been of interest,[45] because of the weak basic properties of the reagent and by-products, which facilitate removal by washing with acid. Peptide synthesis is also claimed to proceed in good yield and without racemisation. Koga and Saski[46] reported the application of thiol esters to peptide bond formation, the key

intermediate being a chiral crown ether having two thiol groups (Fig 2.27) The crown ether acts as a ligand for the

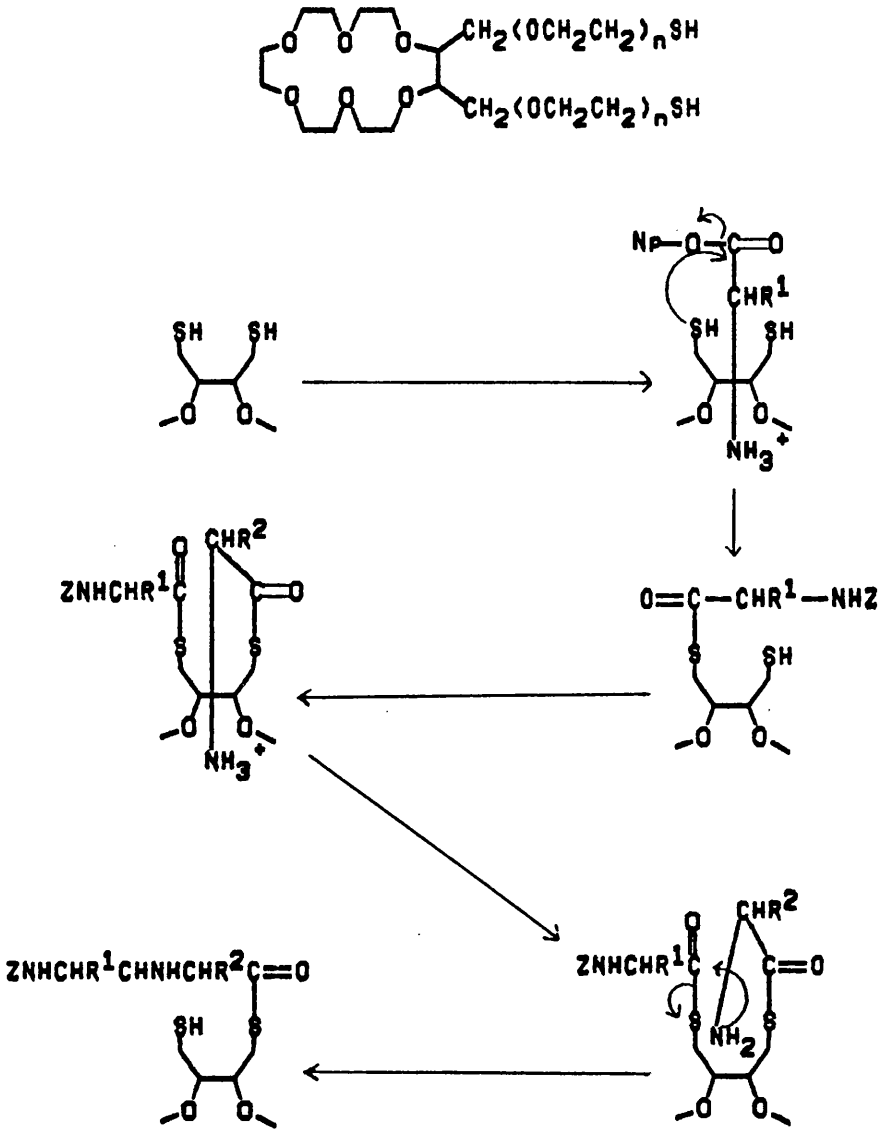

Fig 2.27. a) $^{t}NH_3$-CHR^1-CO_2Np, pH 5.0; b) $ClCO_2Bzl$; c) $^{t}NH_3$-CHR^2-CO_2Np.

NH_3^{t}-group of a 4-nitrophenyl amino acid ester salt. Peptide bond formation takes place in the presence of base to give a monothiol ester of a Z-dipeptide. Unfortunately,

racemisation is high, and appears to be dependant on structure. However this method is of interest, because chain propagation is in the natural direction rather than from the *N*-terminus.

Benoiton et al[47] have reported that mixed anhydrides of t-Boc, Z- and Fmoc-amino acids derived from $ClCO.OCHMe_2$ are fairly stable, and undergo aminolysis with less racemisation than related anhydrides. Reaction between sterically hindered benzimino chlorides and *N*-protected amino acids give *N*-acylamides. The latter afford stereochemically pure peptide derivatives in reasonable yields (Fig 2.28).[48]

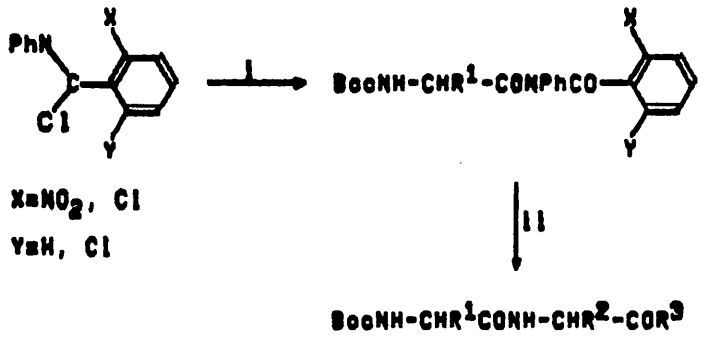

Fig 2.28. i) Boc-NH-CHR^1-CO_2H; ii) NH_2-CHR_2-COR^3

Galpin et al[49] have reported the synthesis of fragments of vasoactive intestinal peptide (VIP), using diphenylphosphinic chloride to form mixed anhydrides. Yields of the stereochemically pure peptides of *N*-methylamino acids were good, when the amino group was protected as a urethane derivative.

Nemoto et al[50] describe the development of a new acyl

anion equivalent, a hydroxymalonitrile (I), for the preparation of "masked active esters", and their use to prepare a dipeptide. The general strategy is illustrated in Fig 2.29. Reaction of (I) with an electrophile gives (III), which can undergo elimination of R, and a cyano group, to produce (II). After demonstrating that (I) showed similar reactivity to active methylene compounds, by reaction with 1-iodoundecane, Nemoto et al[50] synthesised a dipeptide successfully in 61% yield (Fig 2.30).

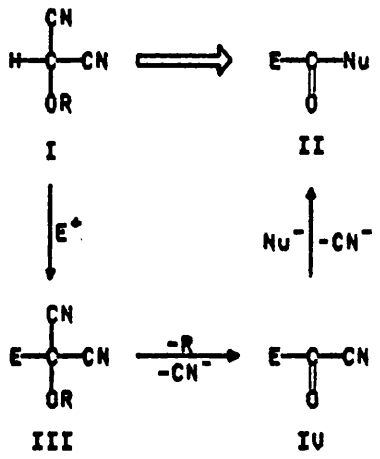

Fig 2.29. Strategy via a masked activated ester; R=-CH_2CH_2-O-CH_2CH_3, $SiMe_2Bu^t$

2.3 Solid Phase Peptide Synthesis.

Using conventional methods, peptide synthesis is often a formidable task, involving the introduction of blocking groups, coupling, deprotection, isolation, and

purification. Hence, the synthesis of peptides containing many residues requires considerable effort by conventional synthesis. The need to automate the process was apparent,

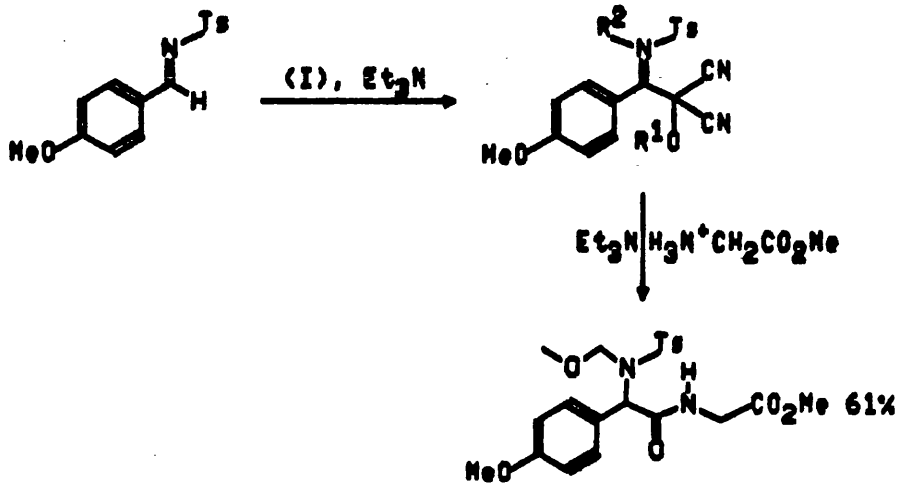

Fig 2.30. Synthesis of a dipeptide; R^1=-CH_2CH_2-O-CH_2CH_3, H; R^2=H, -CH_2OCH_3.

in view of the repetitive nature of chain lengthening using active esters.[51] This was first realised by Merrifield[52] in the synthesis of a tetrapeptide L-leucyl-L-alanyl-glycyl-L-valine. The Merrifield resin, an insoluble support, obtained from the chloromethylation of a styrene-divinylbenzene co-polymer, was developed for this purpose, and remains one of the mainstay of solid phase peptide synthesis (SPPS). The advantages of SPPS is the ease with which the intermediates are obtained free from starting materials, reagents, and most by-products. These can be separated from the desired intermediate,which remains attached to the insoluble polymer, by washing with

appropriate solvents.

The chloromethyl group is allowed to react with the carboxylate form of a protected amino acid (Fig 2.31) to be

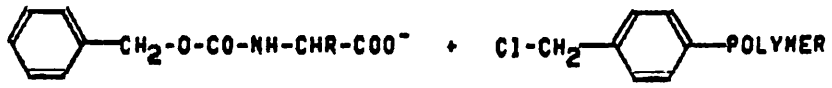

Fig 2.31

anchored via an ester bond. Removal of the benzyloxycarbonyl group with hydrobromic acid gave the aminoacyl resin, which could then be acylated with the next amino acid required in the sequence. However, removal of the benzyloxycarbonyl group under these condition was not entirely selective, and in each de-protection step part of the amino acid or peptide was cleaved from the support and lost. Initially this was circumvented by nitration of the polymer,[53] since nitrobenzyl esters are quite resistant to acidolysis. A further advancement came with the introduction of the use of tert-butyloxycarbonyl group (Boc),[53] since this group can be cleaved with dilute HCl in acetic acid or with a mixture of TFA in dichloromethane (Fig 2.32). Neutralisation of the amine salt with a tertiary amine gave the free amine, which could then be acylated with the next required amino acid residue, protected by a Boc group. In the initial studies by Merrifield, DCCI was used as the coupling agent, excess of which was removed, together with the DCU formed, by washing with dichloromethane (Fig 2.33). This cycle of

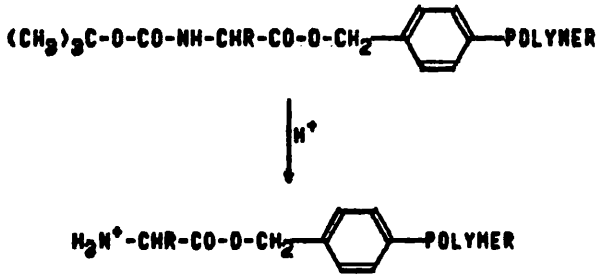

Fig 2.32

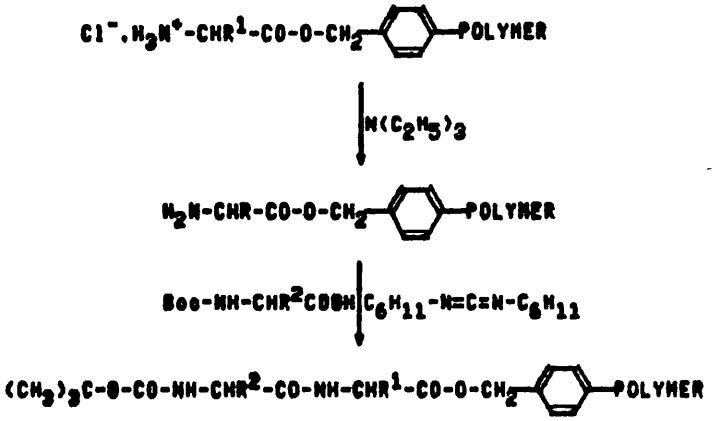

Fig 2.33

deprotection, neutralisation, and acylation is repeated until the desired peptide is achieved, at which point the peptide is cleaved from the support with acid, such as hydrogen bromide in TFA or hydrogen fluoride. (Fig 2.34).

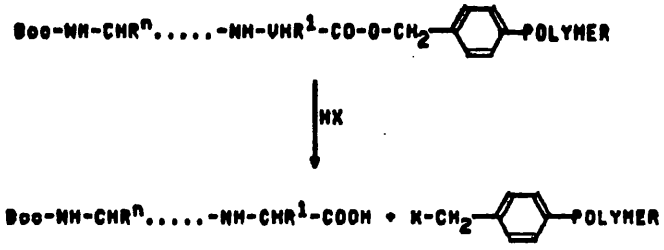

Fig 2.34

2.3.1 Recent development in solid phase peptide synthesis.

Ajayaghosh et al[54] describe a convenient method for the SPPS of C-terminal peptide *N*-alkylamides using a photolytically detachable {[3-nitro-4-({alkyl}methyl)-benzamido)methyl]}polystyrene support (Fig 2.35). The method involves incorporation of an alkylamine moiety into the polystyrene resin, on which the peptides are assembled and subsequently cleaved, by photolysis, in the form of the peptide *N*-alkylamides. The *N*-alkylamino group acts as an anchoring function for the peptide as well as a latent function for the C-terminal modification of the attached peptide. The applicability of the method was demonstrated by the synthesis of *N*-alkylamides (70-77% yields), and analogues of the luteinizing-releasing hormone in 48-56% yield.

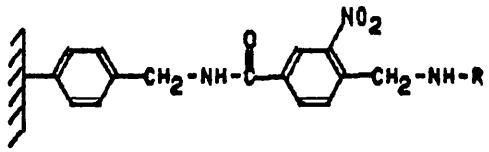

Fig 2.35. R=CH_3, C_2H_5.

Albericio et al[55] describe the preparation and application of 5-[4-(9-fluorenylmethoxycarbonyl)amino-methyl-3,5-dimethoxyphenoxy]-valeric acid (PAL) (Fig 2.36) for the SPPS for C-terminal peptide amides under mild condition. The methodology was demonstrated by syntheses of over a hundred peptides, among which acyl carrier protein (65-74) amide (natural and retro sequences), luteinizing-

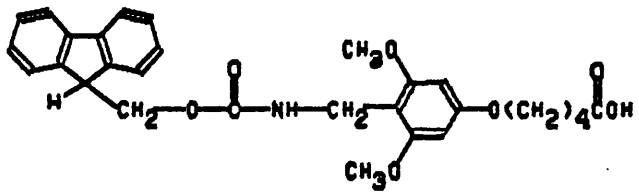

Fig 2.36.

releasing hormone, adipokinetic hormone, PHI porcine fragment (18-27), and human gastrin-I are highlighted. In comparative studies, yields and purities of peptide amides prepared with PAL were found to be equivalent or superior to those found for products prepared by alternative procedures.

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CHAPTER THREE

THE NEED FOR ENANTIOMERIC SEPARATIONS.

3.1 Introduction.

The fundamental concepts of stereochemical optical activity were set forth by Jean-Baptiste Biot[1] in 1815 and Louis Pasteur[2] in 1848, and it is to Pasteur that credit is often given for the first resolution of enantiomers from a racemate by manually separating the non-identical crystals of the sodium salt of tartaric acid (Fig 3.1). These concepts were subsequently included in the general theory

(+) *R,R* Tartaric acid (-) *R,S* Tartaric acid

Fig 3.1. Eanantiomers of tartaric acid; R,S, also known as *meso* tartaric acid.

of organic structure in three dimensions by Le Bel[3] and Van't Hoff.[4] The former postulated a square pyramidal arrangement of substituents around a apical carbon atom, whereas Van't Hoff correctly postulated a tetrahedral

arrangement around a central carbon atom. In one of his lectures before the Council of the Société Chimique de Paris reporting on what is now considered an epochal discovery, Pasteur said, "Most natural organic products, the essential products of life, are asymmetric and possess such asymmetry that they are not superimposable on their images..... Thus we find introduced into physiological principles and investigations the idea of this influence of the molecular asymmetry of natural organic products of great character which establishes perhaps the only well marked line of demarcation that can at present be drawn between the chemistry of dead matter and the chemistry of living matter."[5] It was, however, soon found that the challenge of separating a racemic mixture into its enantiomers often presented difficulties of greater magnitude than the development of a stereospecific synthesis, and as a result, efforts to resolve racemic mixtures were initially very spasmodic. Only very rarely was a successful separation achieved, and then it was often treated as "lucky" or as a result of a tedious process of trial and error, and a systematic approach to the problem was not regarded as being feasible in these early days.

Consequently the technical feasibility of a synthesis was regarded as of greatest importance amongst scientist working on the development of a new drug, and the question of the biological effects, be they desired or undesired, of different stereomchemical forms of the drug molecule were often secondary. Without the availability of chiral high

performance liquid chromatography (HPLC), drug trials were often performed using the racemic mixture rather than the enantiomerically pure drug. The thalidomode tradegy referred to below provides a sad reminder of the consequences of ignoring such considerations.

3.2 Questions Of Clinical Efficacy And Safety.

Enantiomeric molecules only differ in behaviour if the property being investigated also has a characteristic "handedness". For example, enantiomers do not differ in melting point because there is no chirality in the molecular vibrations which increase with increasing temperature, causing the solid to melt. However, the rotation of polarised light does have a specific "handedness" and the enantiomeric molecules thus behave differently with respect to this property. Similarly reaction with a chiral substrate (eg, an enzyme or a chiral HPLC stationary phase) is a process with a "handedness" leading to diastereomeric combinations which differ in properties. All resolutions rely on such differences, even when the diastereomers are only transient in their existence, as in the case of chiral HPLC.

In a physiological environment, ie.,*in vivo*, which is inherently chiral, a molecule of a drug must interact with enzymes, receptors, etc, all of which are asymmetric at the molecular level. Thus enantiomeric molecules would be expected to behave differently under such conditions, but

the differences in physiological effect between enantiomers cannot be predicted.

Thalidomide was not marketed in the USA, but was in Europe. There was no apparent consideration between its biological activity and stereochemistry.[6] Despite the fact that it was claimed to be non-toxic, it was found to be both neurotoxic (causing peripheral neuritis) and teratogenic (causing foetal abnormalities, especially a birth defect, phocomelia, in which the hand or feet started immediately from the shoulder or hip). As a result of the tragedy, research was stimulated into this problem.

A stereospecific synthesis for thalidomide was ultimately developed,[7-9] but initial developmental work was done without the technological tool of chiral HPLC. The racemate was synthesised, at the time of its initial use, in Germany,[10] but a resolution of the racemate into its enantiomers does not seem to have been described in the open literature. The results of the investigations on the safety and efficacy of the stereoisomers of thalidomide suggests that the enantiomers differ significantly in their biological activity. This has led to the conclusion that the teratogenic effects are found in only one enantiomer.

A stereospecific synthesis for thalidomide, starting form glutamic acid or its derivatives[7-9] is available. The absolute configuration of the chiral centre is always known, as the reaction sequence does not involve reaction at the chiral centre. Dextrorotatory thalidomide has the D configuration[9] corresponding to the *R* configuration (Fig

3.2) using the Cahn-Ingold-Prelog[11] nomenclature. A crystal structure has been reported on racemic thalidomide[12] as well as its 4-bromo derivative.[13] The racemate has also been resolved by HPLC.[14]

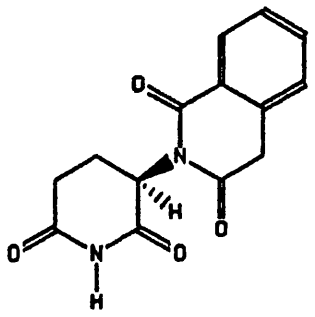

Fig 3.2. (*R*)-Thalidomide

The earliest report into the teratogenic effects of thalidomide[15] at an oral dosage of 150mg/kg from day 7-12 inclusive in New Zealand white rabbits, showed no difference in teratogenic action. The same report also claims that the LD_{50} in SAS ICI albino mice was greater by a factor of approximately twenty times for the racemate relative to the pure stereoisomer. However, a later study[16] showed that the teratogenic activity to be concentrated in the *S*(-)-isomer. This study, however, used SWS mice and Natal rats, rather than New Zealand white rabbits, which are known to be more sensitive to such effects as well as intraperitoneal instead of oral-administration. The effects of thalidomide on graft versus host reaction in chick embryos showed that *S*(-) and the racemic thalidomide had a significant immunosuppressant action which the *R*(+) did

not.[17] The most indicative evidence of the teratogenic action being restricted to *S*(-)-thalidomide comes from studies of its hydrolysis products,[18-22] but this conclusion should be accepted with caution as the mechanism of action remains uncertain. An article by Sumoyi,[23] reviewing an earlier study by Fabro *et al*,[15] noted that the ratio of malformed to normal foetuses was less than half that found for the racemate. The implication is that the pure stereoisomers are mutually synergistic with respect to both toxicity and teratogenicity.

However, none of the studies have succeeded in answering the questions unambiguously and the definitive experiment has yet to be done. The investigations seem to have focused on the stereochemical aspects of the hypnotic and teratogenic effects of thalidomide and neglected its neurotoxicity, also an undesirable side-effect. To assume that the desirable and undesirable action must be separable between the enantiomers may be an over simplification and yet it is necessary to test such a hypothesis using pure enantiomers in order that the necessary experiments are performed.

In the light of the thalidomide tragedy, the US Food, Drug and Cosmetic Act was revised, leading to the establishment of the FDA's authority for evaluating the effectiveness of drugs and applications for investigational studies. Unfortunately routine resolution of racemates was not, at the time, feasible and the Kehauver-Harris Amendments of 1962 contained no requirement as to the

effectiveness of a racemic drug to be evaluated relative to the pure stereoisomers.

3.3 Questions Of Pharmacokinetics.

Within a body, a drug exists in a chiral environment where its release, absorption, transport, action, degradation and elimination may involve interactions with enzymes, cell surfaces, receptor sites etc. This means that the two enantiomers should be acted upon differently by the body. These differences between enantiomers are not limited to pharmacological effects and it has been demonstrated that pharmacokinetic models for the racemic drugs are invalid when assuming a single substrate.

The family of non-steroidal anti-inflammatory drugs (NSAIDs), approved for marketing in the USA are racemic mixtures, except naproxen and its sodium salt. The enantiomers of 2-arylpropionic acids show stereoselectivity in their disposition kinetics,[24-26] and in addition, metabolic inversion of the *R* enantiomer to the *S* form has been demonstrated for many members of this family, tiaprofenic acid being the exception.[27]

Benoxprofen was withdrawn from the market in 1982 when differences in pharmacokinetic behaviour of the enantiomers was discovered. It also exhibited inversion from its *R*(-) to its *S*(-) enantiomer following oral administration in humans,[28] which later studies *in vitro*, indicated to occur as the drug passes through the intestinal wall.[29] The

possibility that the effect of the conversion leading to hepatotoxicity due to the decreased rate of metabolism and excretion in the elderly, must be considered as a contributory factor in its withdrawal from the market.[30,31]

Diisopyramide also shows stereoselective pharmacokinetics, its binding to plasma protein being both stereoselective and concentration dependant.[32] This combination of kinetic factors leads to data which are unexplainable by a model that assumes the drug to be a single component, an assumption which appears to be common practice. In at least one case the enantiomeric ratio has been varied to improve its therapeutic effect.[33] The enantiomers of indeterminate, a long-acting, high-ceiling diuretic, both have uricosuric activity, the (-) enantiomer also being a potent natiurectic agent. A balance between the two natiuretic and uricosuric effects was found for a 4:1 ratio of (+):(-) enantiomers.

The implication is that knowledge of the behaviour of the pure diastereomers can come about through the understanding of the pharmacokinetics of a racemic drug. Given sufficient data about the kinetic behaviour of both enantiomers as well as the racemate, it is tempting to speculate upon the extent to which its pharmacokinetics might vary by using the partially rather than the fully resolved drug.

Improving therapeutic efficacy by using a combination of drugs is not unusual, and FDA regulations require that:- "each component makes a contribution to the claimed effect

and the dosage of each component is such that the combination is safe and effective."

3.4 Investigations Of Pure Enantiomers.

The design of a clinical study, comparing efficacy of one drug with another, is required to be adequate and well controlled, such that the study uses a design that permits a valid comparison, with a control, to provide a quantitative assessment of drug effect. The control drug, in order for the comparison to be valid, should be administered in a clinically effective dose and regimen, but it must also be at a similar dose level and have similar stereochemical composition. Clearly then, comparing the biological effect of a pure stereoisomer with the racemate at the same dose level does not meet this condition.

Consider the case where two enantiomers *R* and *S* are to be compared to the racemate *RS*. If all three were administered at the same dosage in mg/kg, the experimental design, although simple, would be uninformative. If however, both R and S are equal in effectiveness and any synergistic effects are lacking, then an identical response is expected, irrespective of whether *R*, *S* or *RS* was administered, but an unequal response does not lead to an unambiguous conclusion. The conclusion that the biological effect is stereochemically dependant, as a result of a greater effect from *R* or *S* over *RS*, is only correct if a

linear dose-effect relationship is shown and the other enantiomer is totally lacking in activity.

The *R* and *S* isomer should be considered with *RS* at the same level as well as twice the dose (*RS*X2), as the racemate is in a 50:50 composite mixture of the two potentially active substances. Each enantiomer at *RS*X2 is thus present at the same dose level as the enantiomerically pure drug and differences can then be attributed to the presence of the other enantiomer. Different results between *R* versus *RS*X2 and *S* versus *RS*X2 could lead to the conclusion that one of the enantiomers is a stereoisomeric ballast with respect to efficacy or adverse effects. On the basis of such evidence, development of the racemic drug for marketing could not necessarily be excluded, unless an economical stereospecific synthesis was feasible, then the development of the effective enantiomer could be the more preferable option on the basis of efficacy and safety.

If however, both *R* and *S* possessed desirable biological activity to the same degree, the *RS*X2 experiment would show a greater effect, but not necessarily twice as great, as the dose-level would be doubled that of *R*, *S* or *RS*, and providing similar results from studies of toxicity and other undesired effects, then the stereochemically pure drug can be said to have no advantage over the racemate with respect to efficacy and safety. Hence it may be possible to eventually market the racemate using the argument that each enantiomer is optically pure!

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CHAPTER FOUR.

CHIRAL RESOLUTIONS.

4.1 Introduction.

In the past few years there has been a dramatic increase in attempts to use liquid chromatographic (LC) means to resolve non-specific stereochemical problems. The extent to which the use of advanced-level LC apparatus has become routine augurs well for the general acceptance of this rapid, inexpensive and operationally simple method for determining enantiomer purity, absolute configuration, or actually separating useful quantities of stereoisomers.

Improved stereochemical methodology has accompanied the wide acceptance of the technique,[1,2] consequently it is now possible to assess enantiomeric purity accurately for many compounds using analytical high-pressure (HPLC) chromatography, and in many cases absolute configurations have been determined simultaneously, as described in several relevant review articles.[1-10] Whereas analytical-scale technology makes possible the separation of stereoisomers in milligram quantities, gram-scale separations can be effected on preparative, medium-pressure liquid chromatography (MPLC) systems, which can be easily automated. Unlike classical separation methods employing fractional crystallisation, the entrance of a "first-time" separation is frequently predictable for chromatographic

methods.

The separation of enantiomers requires the interaction of a chiral agent. The agent can take the form of a long-term derivatisation of a pair of enantiomers with a chiral derivatising agent (CDA) to afford chromatographically separable diastereomers. This approach requires a discreet derivatisation step and is often termed indirect resolution. For analytical applications the CDA chosen should be of essentially total enantiomeric purity and react non-selectively with both substrate enantiomers to afford stable diastereomers of sufficient separability for the instrument at hand. The cost of the reagent is not especially important owing to the small quantities required. However, indirect resolution methods are not absolute because incomplete enantiomeric purity of the CDA, or different rates of derivatisation of the substrate enantiomers, or non-identical response of diastereomers can adversely influence conclusions regarding substrate enantiomeric purity.

For preparative applications, CDAs should afford diastereomers from which the substrate can ultimately be retrieved. Moreover, the chromatographic separability of the diastereomeric derivatives must be greater (owing to reduced column efficiency), and the cost of the CDAs also becomes a consideration. Different rates of reaction of the enantiomers and different detectability of the diastereomers is not a serious problem, but the overall yield during derivatisation and de-derivatisation should be

high in order to obtain high enantiomeric purity after separation.

Alternatively, separation can be effected via a short-term interaction of the enantiomers with a chiral agent to afford short-lived diastereomeric complexes. This direct resolution method utilises a chiral element on a chromatographic column itself ie, a column that is packed with a chiral stationary phase (CSP). Then the diastereomeric complexes will be of non-identical stability, and the enantiomers will elute at different times. Alternatively a chiral agent may be added to the mobile phase, a chiral mobile phase additive (CMPA), with the intention that the diastereomeric complexes will differ either in stability or in chromatographic behaviour on an achiral column. Direct chromatographic resolution methods are free from a number of restraints placed on indirect methods. In particular direct methods are absolute in the sense that no external standard of enantiomeric purity is needed to determine accurately the enantiomeric purity of a substrate. Chromatography, like nuclear magnetic resonance (NMR), gives a weighted time-average view of the dynamic process. Therefore, a less than enantiomerically pure CSP or CMPA affects the position but not the relative size of the two bands stemming from the solute enantiomers. Reduction of the enantiomeric purity of a CSP or CMPA diminishes α (see section 4.4) by increasing k_1 and k_2 until, at the racemic limit, the two values become identical. If a column packed with a CSP of 80%

enantiomeric purity is used, the column will still function effectively, although the observed α-1 values will be *ca* 80% of the maximum attainable. From a preparative standpoint, direct resolutions may afford higher overall yields, because derivatisation and de-derivatisation may be unnecessary. However, derivatisation is sometimes employed either to enhance detectability or to provide functionality necessary for the separation process. Since derivatising agents employed are achiral, no significant possibility exists for the selective derivatisation of one enantiomer and incomplete derivatisation is of little matter for analytical applications.

Offsetting the overall advantage of the direct method is the fact that commercially available CSP and CMPAs are expensive, but a chiral column can be used a great many times, thereby compensating for the initial expense or difficulty in obtaining the column. The CMPA requires an ongoing source (or recovery) of the chiral additive. This problem should be alleviated with the advent of micro-bore analytical columns giving reduced mobile phase consumption, but will remain a serious drawback for preparative separations. Moreover, the CMPA must ultimately be separated from each enantiomer after a preparative resolution.

As in the CDA method, the responses to diastereomers from the CMPA method need not be identical, whereas in the CSP approach identical responses are detected for the enantiomers.

Absolute configuration can be determined by many chromatographic methods if a sample of known configuration is available for comparison. Failing this, absolute configuration can be assigned on the basis of elution order, provided that the absolute configuration of the chiral agent and the mechanism of chromatographic separation of the relevant stereoisomers is known. An additional advantage of the direct method is that the covalent diastereomers may differ in their NMR parameters and it may be possible to correlate these differences with relative and hence absolute configuration. Short-lived diastereomeric solvates can also differ in their NMR parameters and these differences may also be correlated to relative/absolute stereochemistry in favourable cases

4.2 Historical Background.

In 1904 Wilstätter[11] postulated that it should be possible to resolve racemic dyes from solution by enantioselective adsorption onto wool. Partial resolution of an optically active aniline on wool was subsequently demonstrated by Ingersoll and Adams,[12] and then by Porter and Ihrig.[13] Although attempts to repeat some of these results using wool were unsuccessful,[14,15] Porter and Ihrig's dyes were found to be partially resolved using powdered silk or potato starch as the chromatographic adsorbents.[16] In further earlier studies by Henderson and Rule,[15] resolution of a racemic camphor derivative and of Tröger's

base,[17] using lactose as the adsorbent, provided conclusive experimental verification of Wilstätter's idea.

The classical method for the resolution of enantiomers involves the reaction of the racemate with an optically active reagent to form a pair of diastereomers which have different physical properties. The diastereomers can then be separated by fractional crystallisation or chromatography and the enantiomers recovered. However, this method is not without problems. The method requires that a suitable functional group be available in the racemic substrate as well as sufficient quantities of an optically pure reagent of suitable structure. In addition there is the problem of racemisation during diastereomer formation or retrieval of the individual enantiomers. In contrast, direct chromatographic methods do not rely on prior formation of diastereomers.

The resolution of enantiomers using an optically active stationary phase, coated onto a 100m gas chromatography (GC) capillary column, was first achieved by Gil-Av *et al.*[18] However, GC has the disadvantage of using higher operating temperatures, compared to HPLC, which may lead to racemisation of both the chiral stationary phase (with concomitant loss of chiral selectivity) and of the enantiomers to be separated. In the chromatographic methods, the covalent bond of a true diastereomer is replaced by the transient interaction between the optically active component (chiral selector) and the enantiomers of the racemate. If one enantiomer interacts more strongly

with the chiral selector, then it will be more strongly retained on the column, and hence chromatographic selectivity is achieved.

Two decades ago, the direct resolution of enantiomers was rare, whereas now, there is an abundance of different LC solutions to this problem. This is due to several factors such as the break through in HPLC technology in the late 1960's, together with insights gained in the chiral resolution mechanism by Pirkle and others and the advancement of HPLC hardware (pumps, low dead-volume, injectors and detectors). These enabled fast and efficient LC, and of particular sigificance were the advances made in HPLC packing materials used as the basis for CSPs. Polystyrene and cellulose resins were not suitable for use under the high operating pressures of HPLC, plus their mass transfer characteristics were poor. These problems were overcome with the advent of mechanically stable, superficially porous supports in which a thin layer of silica was grafted onto the surface of glass beads, thereby reducing diffusion distances. The most significant advances resulted from the availability of small diameter, porous silica particles of re-producible dimensions (3-5μm), and with these packing materials, modern phases, such as silica bonded cellulose, protein and synthetic chiral selectors became possible. HPLC's increasing popularity in the 1970's has been well documented.[19,20]

4.3 Theoretical Background.

Senoh and co-workers[21] reported one of the earliest examples of enantiomer separation on a CSP. They noted that aromatic amino acid enantiomers could be separated on paper, an adsorbent which is chiral both due to the glucose component and by virtue of the helicity of the cellulose polymer. An extension of this work by Dalgleish[22] foreshadowed the "three-point rule" for chiral recognition.

Dalgleish noted that derivatisation of the amino or carboxylate group or removal of the aromatic funtionality of the amino acid resulted in loss of separation and he concluded that three simultaneous interactions were necessary between the adsorbent and the enantiomers for enantioselectivity to occur. "Chiral recognition requires a minimum of three simultaneous interactions between the CSP and at least one of the enantiomers, with at least one of these interactions being stereochemically dependant."[2] This requirement can be shown as in Fig 4.1. The two enantiomeric structures can be "recognised" by the chiral selector if at least three active positions of that agent (A', B' and C') simultaneously interact with the appropriate active positions (A, B and C) of one enantiomer. In this case the corresponding positions of the second enantiomer would appear in the wrong sequence (A, C and B) in respect to the chiral selector. This results in a difference in the total formation energy of the two diastereomeric adducts resulting in discrimination of the two enantiomers. Thus according to the "three-point rule",

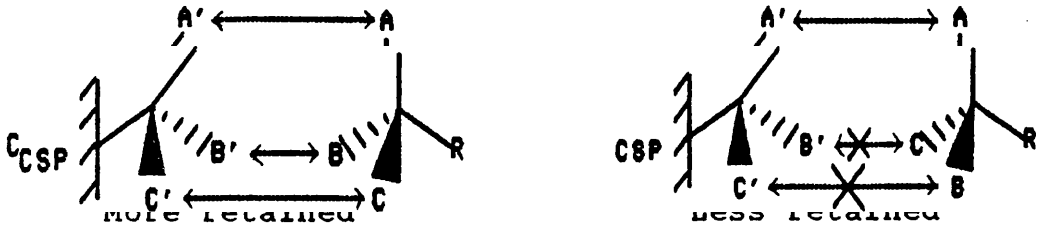
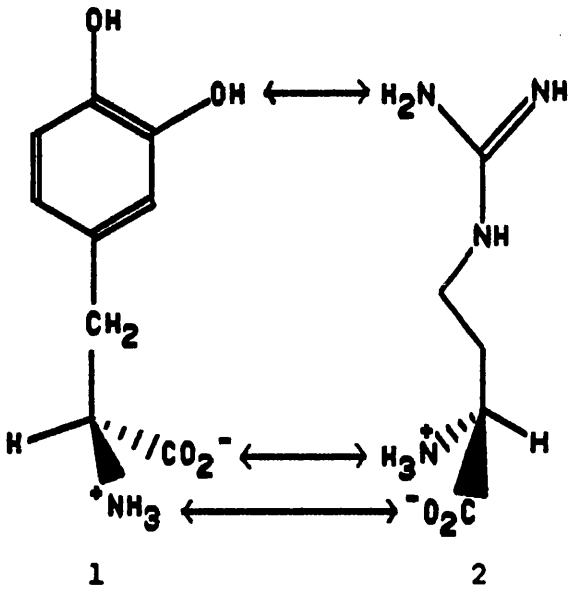


Fig 4.1. Three-point rule for chiral recognition. The more retained enantiomer is capable of three simultaneous interactions (A-A', B-B' and C-C') with the CSP, whereas the less retained is capable of only two interactions with the CSP.

any combination of three attractive or repulsive interactions in the vicinity of the chiral centre of the molecule could lead to chiral recognition of its enantiomers, provided that chromatographic conditions favour simultaneously, all interactions resposible for enantionselectivity.

The first practical application of the three-point rule to the design of CSPs was by Baczuk *et al.*[23] Using space filling models, Baczuk suggested that l-arginine might interact with the l-enantiomer of dihydroxyphenylalanine, DOPA 1 (Fig 4.2) with three simultaneous electrostatic interaction, whereas for l-arginine and d-DOPA, only two simultaneous interactions were possible. l-Arginine 2 was covently bonded to Sephadex resin to separate the enantiomers of DOPA. Surprisingly, it was the d-enantiomer of DOPA that was most retained, indicating that the mechanism of chiral recognition was not that originally postulated.



1 2

Fig 4.2. Three point interaction proposed by Baczuk to explain the separation on enantiomers DOPA 1 on a CSP derived from l-arginine 2.

There have been suggestions that three simultaneous interactions are not always necessary. The three-point rule was modified by Davankov and Kurganov[24] for certain cases where only two points of interaction between the adsorbate enantiomer and the immobilised chiral agent are necessary, with the extent of adsorption of the diastereomeric complexes on the achiral surface of the stationary support determining selectivity. This is considered further below, suffice to say here that the spatial orientation of the adsorbed species with respect to the achiral surface and the extent of the adsorption are still determined by the chirality of the moiety attached to the surface, and therefore Davankov's case is an extension of and not an exception to the three-point rule. The separation of *N*-acyl

amino acid ester enantiomers on CSPs derived from N-acyl amino acids in terms of two interactions (head-to-tail hydrogen bonding between the N-H and C-terminal carbonyl oxygens of the CSPs and analyte), was discussed by Hara and co-workers[25,26] (Fig 4.3). Unfortunately neither enantiomer of the analyte would be capable of forming these hydrogen bonds, and Hara's proposal is insufficient to account for chiral recognition. Pirkle[27] however, proposed a face-to-face rather than an edge-to-edge approach. This "stacking"

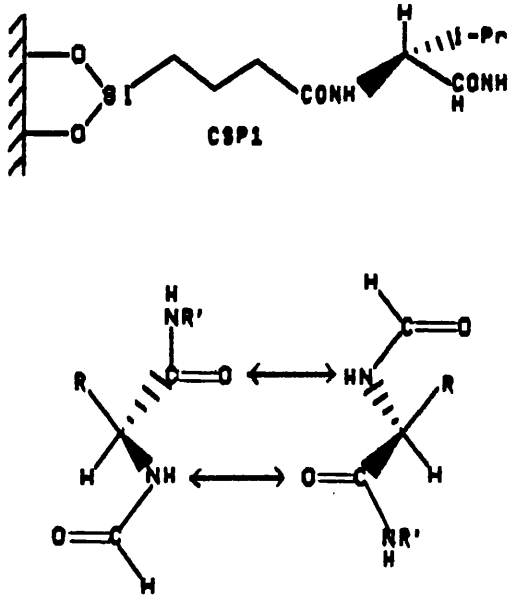


Fig 4.3. Hara's CSP 1 is capable of separating the enantiomers of acylated a-amino acid esters and amodes. He proposed a two point hydrogen bonding model to explain the separation.

attributed to dipole alignment between the analyte enantiomers and the CSP, explains the sense of enantiomer separation seen and is consistent with the three point rule

(Fig 4.4). "Stacking" of the dipoles is functionally equivalent to a two point interaction, but with a single additional interaction which suffices to afford chiral recognition. Similar arguments can be adduced for π-π interactions between aromatic rings. Hara[28] has recently considered additional interactions with the silica support as a possible explanation of the chiral recognition observed.

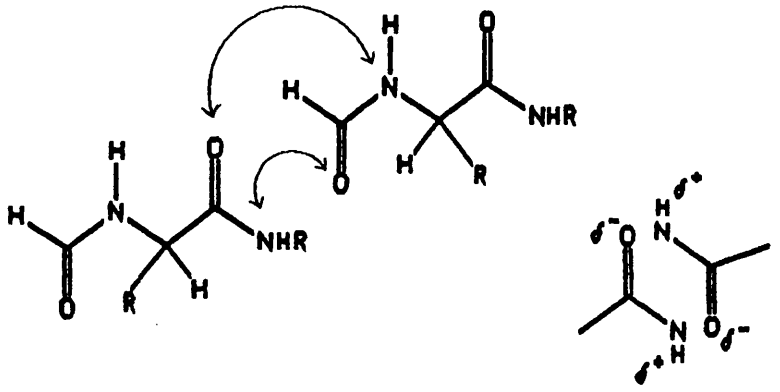


Fig 4.4. Pirkle's proposed dipole stacking, which is face-to-face and gives a net four points of interaction between th CSP and analyte, as the mechanism of chiral recognition on Hara's CSP 1.

4.4 Chromatographic Terms.

A few chromatographic terms should be defined for readers who are not experienced with chromatography. The volume of the solvent required to elute a non-retained solute is equal to the void volume of the column. An additional volume of solvent is then required to elute a retained solute. The ratio of this additional volume of

solvent to the void volume is the capacity ratio k. The separability factor α, for the two solutes is k_2/k_1, a volume which corresponds to the ratio of the two partition coefficients and is related to the energy difference between the retention mechanism of the two solutes (Fig 4.5). The degree of separation of the two components depends on both k_1 and k_2 and on column efficiency, which is a band shape consideration and is determined by factors such as column packing skills, particle size, samples size

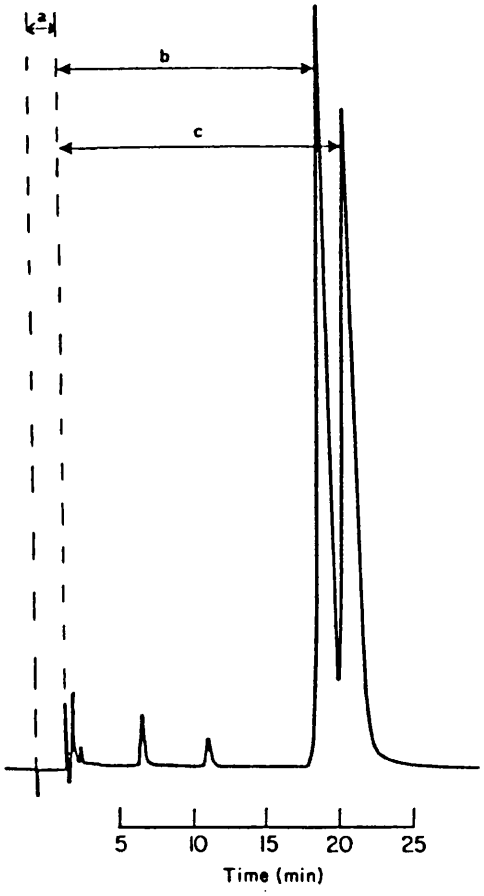


Fig 4.5. Chromatogram illustrating the terms capacity ratio (k_1=b/a; k_2=c/a) and separability factor ($\alpha=k_2/k_1$).

and flow rate. For a given column, the magnitude of α is the most important factor for the separation of two solutes. As in both analytical and preparative resolutions, total separation of stereoisomers is desired, so particular attention is paid to the magnitude of α for the stereoisomers under discussion. Reasonably good separations of two solutes having an α of 1.04 or greater is often observed for highly efficient analytical HPLC systems. MPLC systems typically use larger particle size-packings than HPLC systems and consequently preparative MPLC systems have reduced efficiency and α values of 1.20 or greater are required for effective resolution of sizable materials.

4.5 Thermodynamic Parameters.

As already mentioned in the previous section, the separation factor, α_{DL}, is the ratio of the capacity ratios of the D and L enantiomers, k_D and k_L, for a given set of chromatogaphic conditions (Eq 4.1). As capacity factors are

Eq 4.1 $$\alpha_{DL}=k_D/k_L$$

equilibrium constants Eq 4.1 can be re-written as Eq 4.2

Eq 4.2 $$\alpha_{DL}=\frac{\exp(-\frac{\Delta G_D^{\circ}}{RT})}{\exp(-\frac{\Delta G_L^{\circ}}{RT})}$$

where ΔG^{a}_{i} are the molecular free energies of adsorption. Re-arrangement of Eq 4.2 gives the relationship between α_{DL} and δG^{a}, the difference in molar free energy of formation of the diastereomeric complexes (Eq 4.3). δG^{a} is related to

Eq 4.3 $$\delta G^{a} = -RT \ln \alpha_{DL}$$

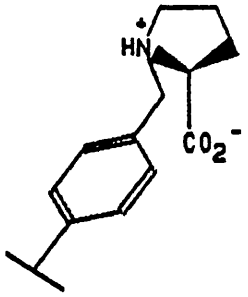
the difference in enthalpy and entropy of adsorption by Eq 4.4. δG_{a} may be non zero due to either the entropy or enthalpy terms of Eq 4.3. By monitoring α as a function of

Eq 4.4 $$\delta G^{a} = \delta H^{a} - T\delta S^{a}$$

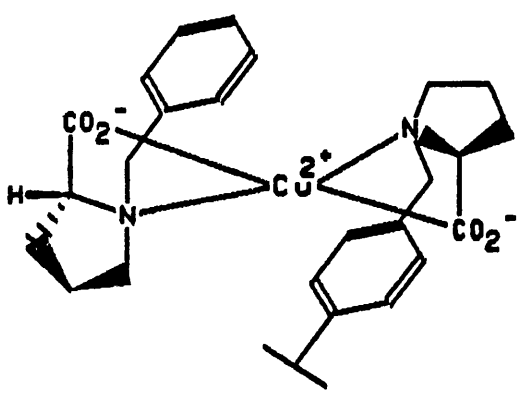
temperature, it is possible to determine whether entropy or enthalpy differences control enantiomer separation. Relatively small values of δG^{a} ($\approx$200J) are sufficient to afford observable chromatographic separations (α>1).

In the majority of cases the separation of enantiomers on a CSP is enthalpy-dominated, if three simultaneous interactions between the stationary phase and the analyte enantiomer occur, and at least one of them is stereochemically dependant, then separation will occur, with the enantiomer forming the most stable complex with the stationary phase being the more retained. The importance of the enthalpy term in Eq 4.4 for enantiomer separations explains the small separation factors typically observed on chiral GC stationary phases (high operating

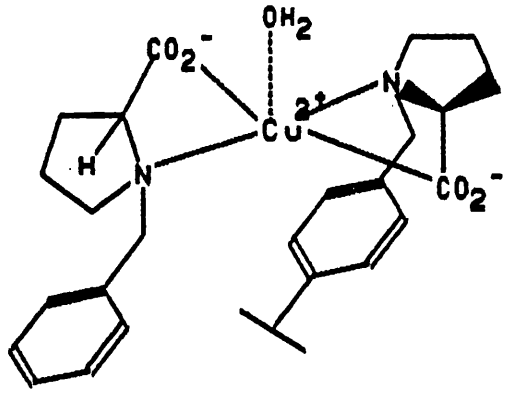
temperatures increase the relative importance of the enthalpy term, causing a decrease in δG_a). Only one case of entropy controlled enantiomer separation has been reported, and this special case comes about because differing numbers of species are involved in the formation of the diastereomeric complexes.[29] In such cases entropy changes are often a controlling factor in chemical equilibria.[30] Davankov, who reported this instance, noted that the enantiomers of *N*-benzylproline were separated on CSP 2, derived from l-*N*-benzylproline, in the presence of Cu^{2+} and that the degree of separation increased slightly with temperature, indicating entropy control. It was hypothesized that the diastereomeric complexes formed between the enantiomers of the analyte, Cu^{2+}, and the fixed l-*N*-benzylproline moiety of the CSP contained different numbers of ligands. In the *l,l*-complex, a solvent molecule (water), may form an axial ligand to the Cu^{2+}, while steric hindrance, resulting from the positioning of the *N*-benzyl group in the *d,l*-complex, prevents this additional bonding interaction. It is clear that the *d,l*-complex, involving fewer ligands, is favoured entropically (Fig 4.6). At temperatures close to ambient, the entropy term is larger than the enthalpy term in the free energy Eq 4.3, for this separation. This example does not, however, contravene the three point rule, since three interactions between the two chiral species involved in the separation, whether direct or mediated by an axial ligand, are still seen. Whether the enantiomer separation is enthaply- or



CSP 2



d,l



l,l

Fig 4.6. Entropy control of δG during the separation of *N*-benzyl-proline enantiomers on CSP 2. The more favoured diarstereomeric complex (*d,l*) has fewer species (two) involved in its formation and hence is favoured entropically over the (*l,l*) complex. As expected, separation factors increase slightly with temperature.

entropy-controlled, it must be concluded that the three point rule remains valid.

One can also imagine situations in which direct three-point interaction between chiral centres may not be necessary for a degree of stereo-selectivity to occur. Solution aggregates of chiral molecules might be expected

to display differences between the homochiral and heterochiral aggregates based solely on symmetry considerations alone.[31] These considerations, however, are not expected to apply to CSP-analyte interactions, because the CSP and analyte can never occur, and the above argument will not apply to separations on CSPs. For solutions of racemates, such effects will cancel out, since equal amounts of the *meso* and enantiomeric forms will be present. On the other hand, effects might be noticeable for enantiomerically enriched samples during achiral chromatography (leading to enrichment of fractions in one enantiomer)[32] or nuclear magnetic resonace self-induced non-equivalence studies.[33]

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CHAPTER FIVE.

INDIRECT RESOLUTION OF ENANTIOMERIC MIXTURES.

5.1 Introduction.

A variety of approaches to the chromatographic separation of enantiomeric mixtures have been reported.[1,2] Among these, indirect methods are based on the reaction of a racemic mixture with a chiral reagent to form a pair of diastereomers as described in the previous chapter:-

(R)-A + (R,S)-B --> (R)-A(R)-B + (R)-A(S)-B

These diastereomers possess different physicochemical properties and can generally be separated by GC, HPLC or on non-chiral columns. Although the indirect methods have been extensively used in the past, eg., in the analysis of enantiomers of β-blockers,[3] they have certain disadvantages:-

1) they are time-consuming, tedious and require optically pure (and hence expensive) derivatising agents, since the enantiomeric contamination of the reagents could lead to false determinations.[4]

2) they often fail to accomplish total separation of the enantiomers.[5] As the resulting diastereomers have different physicochemical properties, the rate of formation may not be the same for each member of the pair. This may result in the generation of two diastereomeric products differing in properties from the starting enantiomer.[6]

3) a further chemical treatment is necessary if the starting enantiomers are to be recovered.

5.2 Scope and Chemistry.

The chiral substances most commonly resolved as diasteromers are amines, alcohols and carboxylic acids, the main types of derivatives formed being:-

AMINES:- amides, carbamates, thioureas and ureas;

ALCOHOLS:- carbamates, carbonates and esters;

CARBOXYLIC ACIDS:- amides and esters;

Amines are the easiest to derivatise, the amino group being the site for derivatisation in compounds such as amino acids and amino alcohols. Amines, alcohols and carboxylic acids also act as chiral pre-cuurors for chiral derivatising agents, eg., isocyanates are prepared from the action of phosgene and the corresponding hydrochloride salt of the amine, and chloroformates are prepared from the action of alcohols with phosgene.

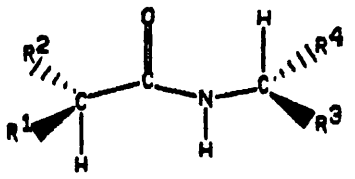
Derivatisation should be in high yield to prevent changes in the enantiomeric ratio and ensure good accuracy.

5.2.1 Diastereomeric amides, carbamates and ureas.

Helmchem and co-workers[7-11] have focused on the separation of diastereomeric amides 1 (Table 5.1) derived from acids (or lactones) and amines. One of the reaction partners is used as the chiral derivatising agent (CDA) and must be available as a single enantiomer. Helmchem has

separated a wide variety of diastereomers on silica gel, the reported α values (**Table 5.1**) being significantly large

Table 5.1. Chromatographic separation of diastereomeric amides.



1

R^1	R^2	R^3	R^4	α^a
Et	Ph	Me	α-Naphthyl	1.80
Me	Ph	Me	Ph	1.81
Me	nBu	Me	p-$NO_2C_6H_4$	2.21
Me	CH_3Ph	CH_2OH	Ph	2.56
CH_2OH	Ph	Me	Ph	2.81
$(CH_2)_3OH$	Ph	Me	Ph	2.13

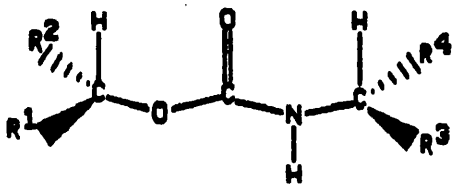
a:- The diastereomer with the relaive configuration shown is eluted last.

for straight forward preparative resolutions. Subsequent retrieval of the chiral acid can be complicated by racemisation during hydrolysis. Helmchem typically used acid-promoted hydrolysis and reports that amides containing appropriately situated hydroxyl groups will hydrolyse under

mild conditions without racemisation of the acid component.

A series of diastereomeric carbamates has been examined by Pirkle and co-workers.[12-15] The carbamates 2 (Table 5.2) are derived either from alcohols and isocyanates or from chloroformates and amines. In each case

Table 5.2. Chromatographic separation of diastereomeric carbamates.



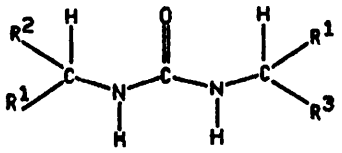
R3=α-Naphthyl; R4=CH3
2

R^1	R^2	α^a	R^1	R^2	α^a
CF_3	Ph	1.58	Me	$(CH_2)_4CN$	1.4
CF_3	α-Naphthyl	1.56	$C{\equiv}C(CH_2)_8CH_3$	$(CH_2)_3CN$	1.8
$n\text{-}C_3F_7$	Ph	2.12	$CH_2SC_6H_5$	Et	1.16
Ph	Me	1.30	$CH_2SCH_2CH_3$	n-Octyl	1.17
Et	Ph	1.22	C≡CH	Et	1.21
t-Bu	α-Naphthyl	1.31	C≡CH	n-Hexyl	1.43

a:- The diastereomer with the relative configuration shown above is eluted last.

one of the components is the CDA and must be available as a single enantiomer. Table 5.2 provides α values for a number of carbamate diastereomers, these values generally being adequate for facile preparative separation on silica or alumina columns. After separation, enantiomerically pure alcohols can be retrieved from the carbamates by the use of trichlorosilane,[16] a reagent that functions under mild conditions in a variety of solvents and that tolerates the presence of a number of other functional groups in the carbamates.

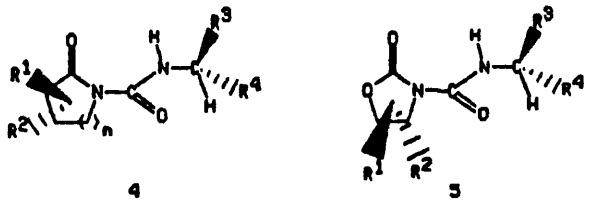
Although a number of diastereomeric ureas are known to be chromatographically separable, no systematic study of ureas such as 3 has been reported. Such ureas are usually rather insoluble, quite polar and, if hydrolysed, would give two amino components. Unsymmetrical acylureas such as



3

4 and 5 have been studied and found to be resolved quite well (Table 5.3) in a systematic manner.[17,18] These compounds, derived from the action of isocyanates on lactams 6 and oxazolidinones 7 can be derivatised smoothly. This method offers great promise for the resolution of a number of heterocycles. Alternatively, oxazolidinones CDA's such as 8 can be used to resolve amines.

Table 5.3. Chromatographic separation of diastereomeric urea derivatives 4 and 5.



4 5

R^2=H; R^3=α-naphthyl; R^4=Me

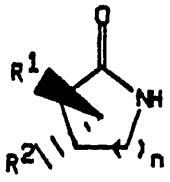
Derivative	R^1	α^a
4a	5-(C_6H_5)	2.29
4b	5-(p-FC_6H_4)	2.47
4c	3-(C_6H_5)	1.84
5a	4-(C_6H_5)	2.05
5b	5-(C_6H_5)	2.32
5c	5-(α-naphthyl)	2.61

a:- The diastereomer with the relative configuration shown above is eluted last.

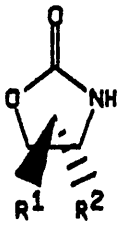
5.2.2 Mechanism of chromatographic separation.

Two substances separate chromatographically owing to a blend of differential solvation by the mobile phase and differential adsorption by the stationary phase. Nuclear magnetic resonance (NMR) and infrared (IR) studies of the aforementioned amides, carbamates and ureas strongly indicate that these compounds have a more or less semi-

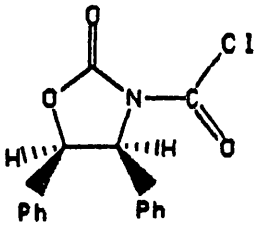
rigid planar backbone composed of all the atoms in 1-5 not indicated to project from the page. Extensive population of the conformations shown in 1-5 (Tables 5.1-5.3) stem from combinations of hydrogen bonding, dipolar repulsion, steric and carbonyl hydrogen bonding effects. Owing to multiple-



6



7



8

site binding of the polar backbone to the adsorbent, the backbone lies more or less flat on the adsorbent with the two substituent projecting from the "bottom-face" (Fig 5.1)

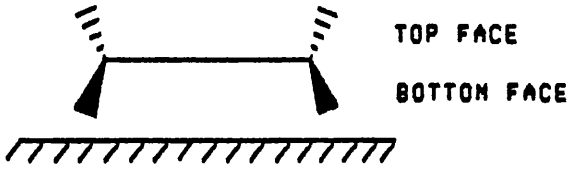


Fig 5.1

of the backbone directed towards the adsorbent. The "top-face" substituents project away from the adsorbent. The "bottom-face" substituents provide important interaction

with the adsorbent, which may be either repulsive or attractive. The relative disposition of the substituents (eg, whether R^1 is *syn* or *anti* to R^3 in the depicted conformations) coupled with the extent and sense of their interactions with the adsorbent determines the elution order of the two diastereomers. Differential solvation seems important. When R^1 is more "repulsive" towards the adsorbent than R^2, and R^3 is more "repulsive" than R^4, the diastereomer having R^1 *syn* to R^4 will be more strongly retained. Alternatively, if R^1 is more "attractive" than R^2, and R^3 is more attractive than R^4, the diastereomer having R^1 *syn* to R^3 will be the more strongly retained. By using such simple concepts and ranking the interactive capacity of the substituent, one can correlate stereochemistry and elution order. The actual magnitude of interaction of a given substituent with the adsorbent depends on the adsorbent, other substituents present and the type and rigidity of the backbone itself. Although no serious attempts at quantification have been made, repulsive interactions toward silica and alumina can be ranked approximately as H < methyl < phenyl ≈ ethyl < propyl < *tert*-butyl < trifluoromethyl < α-naphthyl ≈ pentafluoroethyl < heptafluoropropyl. Size and hydrophobicity are both relevant; incorporation of polar functionality (hydroxyl, carboxy, cyano) leads to attractive rather than repulsive interactions with the silica or alumina.

The drawings shown in Tables 5.1-5.3 represent the

stereochemistry of the more strongly retained diastereomer. The NMR spectral differences between diastereomers can sometimes be used to check stereochemical inferences drawn from the elution orders. In general, an aryl substituent will more heavily shield a *syn* than an *anti* substituent.

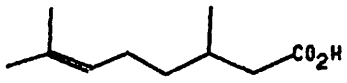
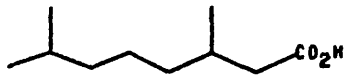
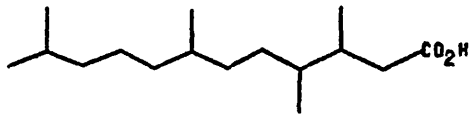
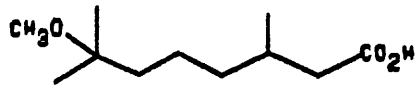
It should also be noted that the chiral acyclic subunits 1-5 bear a single hydrogen on the chiral centre. The presence of this hydrogen confers a degree of conformational control (essential to separation), which may not be present if some substituent other than hydrogen is present. However, if conformational rigidity is still present, separation of the diastereomers might still occur for essentially the same reasons. It is also important that the chiral amine subunits 1-5 be primary so that the (Z)-amide rotamer is preferentially populated. Diastereomers derived from secondary amines have been observed to separate, but with diminished α values.

5.3 Applications.

Subject to the limitations in Chapter 4, the chromatographic separation of diastereomeric amides and carbamates can be used to assay enatiomeric purities and assign absolute configuration for a variety of alcohols, acids and amines. In applying this method to chiral acids, diastereomeric amides derived form 1-(phenyl)ethylamine[19,20] or 1-(α-naphthyl)ethylamine[21] are typically used. Several research groups,[22-24] have applied this method to a series of

isoprenoid and terpenoid acids using (*R*)-1-(p-nitrophenyl)-ethylamine or 1-(α-naphthyl)ethylamine as CDAs (**Table 5.4**).

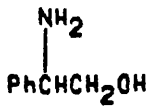
Table 5.4. Resolution of chiral isoprenoid acids via the chromatographic separation of a diastereomeric amide derivative.

Acid component	amine component[a]	α
	A	1.22
	B	1.21
	A	1.21
	B	1.24
	A	1.05
	B	1.09

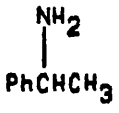
A:- 1-(p-Nitrophenyl)ethylamine; B:- 1-(α-Naphthyl)ethylamine.

It is important to note that sizeable α values are observed even though the chiral centre of the acid is separated by a methylene unit from the amide functionality. The chromatographic separation of diastereomeric hydroxyamides has been utilised to obtained optically active acids and amines on a preparative scale. Helmchem *et al*[9] have shown that the hydroxyamides exhibit very favourable chromatographic properties (**Table 5.1**) and are hydrolysed under mild non-racemising cond itions, overall resolution yields typically being 80-90%. (*R*)-Phenylglycinol **9a** is suggested as a CDA for the resolution of acids whereas 1-(phenyl)ethylamine **9b** is suggested for the resolution of lactones such as **10a** and **10b**. Conversely, amines can also be resolved by using lactone **10c** as a CDA. The observed elution order for all hydroxyamines examined by Helmchem are those expected on the basis of the described chromatographic mode. Hence, relative/absolute configurations of the resolved components can be assigned.

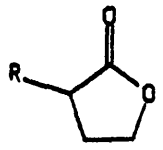
Ade and co-workers[25] have utilised the chromatographic separability of diastereomeric hydroxyamides in the synthesis of the optically active hydrocarbon **11**, the pheromone of the tsetse fly. In this synthesis diastereomeric amides **12** were separated by chromatography and then hydrolysed. Due to the relatively large α value, the diastereomeric amides were readily resolved in 3g lots on a medium pressure liquid chromatography system (MPLC) (**Fig 5.2**). The optically active acid was subsequently converted to the pheromone by standard methods.



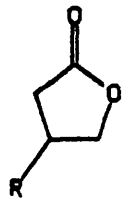
9a



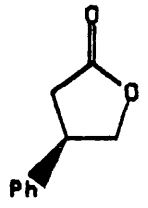
9b



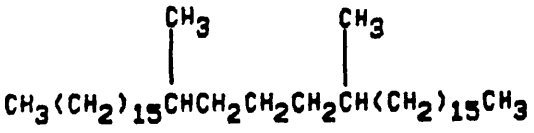
10a



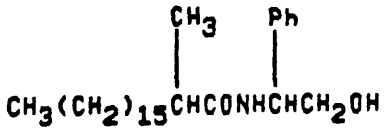
10b



10c



11



12

Enders and Louters[26] demonstrated that diastereomeric α-hydroxyamides 14 can be easily separated with α values ranging between 1.5 and 2.0. The resolved α-hydroxyamides are versatile intermediates because they can be converted into optically active α-hydroxyketones 15, diols 16 or α-hydroxyacids 17 (Scheme 5.1). A chromatographic model correlating elution orders and α values with stereochemistry and structure was not proposed.

A number of amino acids have been indirectly resolved by HPLC. Generally, the CDA is used to derivatise the amino group, the carbonyl group being previously converted to an ester. Of the derivatives examined, amides[27] and thioureas[28]

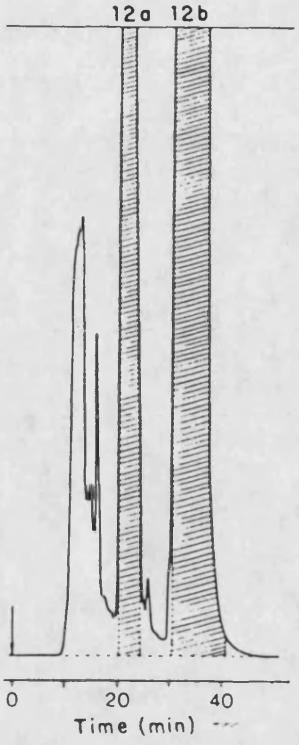
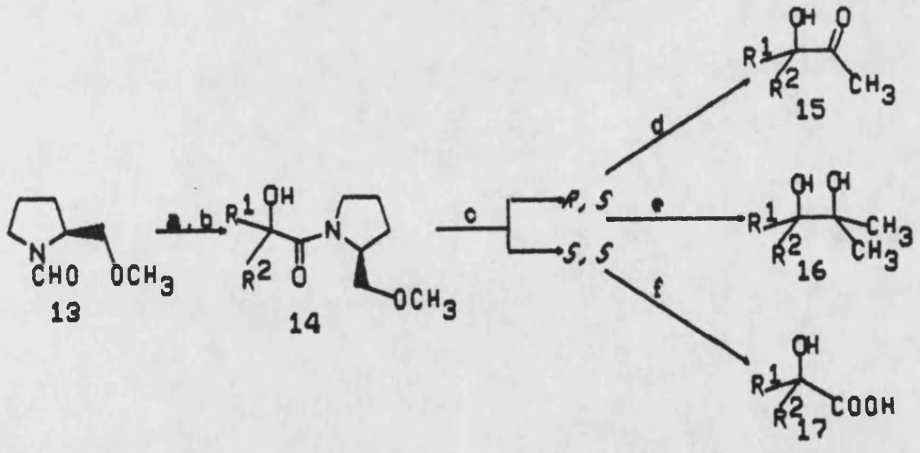
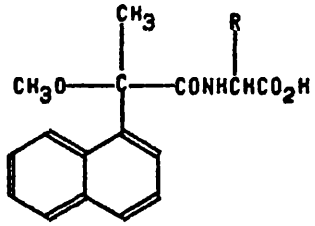


Fig 5.2. Preparative LC separation of the diastereomeric amides 12a and 12b.



Scheme 5.1. a, LiTMP, -100°C; b, R^1COR^2; c, chromatography; d, 1eq MeLi; e, 2eq MeLi; f, conc HCl.

such as 18-20 seem to be the most suitable. Although the amide derivatives are the most readily separated, the isothiocyanate CDAs are more readily accessible, and the thiourea chromophore facilitates ultraviolet detection. Chromatographic separability of diastereomeric carbamates



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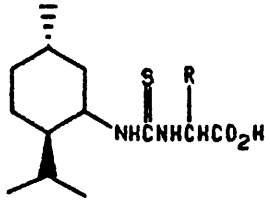

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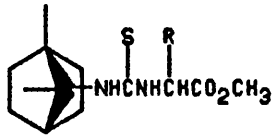


20

has proved to be a useful and general method for preparatively resolving secondary alcohols. Typically, carbamates derived from either 1-(α-naphthyl)ethyl isocyanate 21a or 1-phenylethyl isocyanate 21b are used. The use of 21a usually leads to higher α values, but 21b is much less expensive. Pirkle and co-workers[16,29] and Finn[30] have used this method to resolve a series of trifluoromethyl carbinols 22a-22d, which are of value as chiral solvating agents and used for precursors of CSPs. Fig 5.3 shows the chromatogram for the automated repetitive resolution of 1.0g-samples of the carbamate derived from 22d and CDA 21a. By the use of larger scale operations,

hundred gram quantities of the resolved carbinols have been obtained.

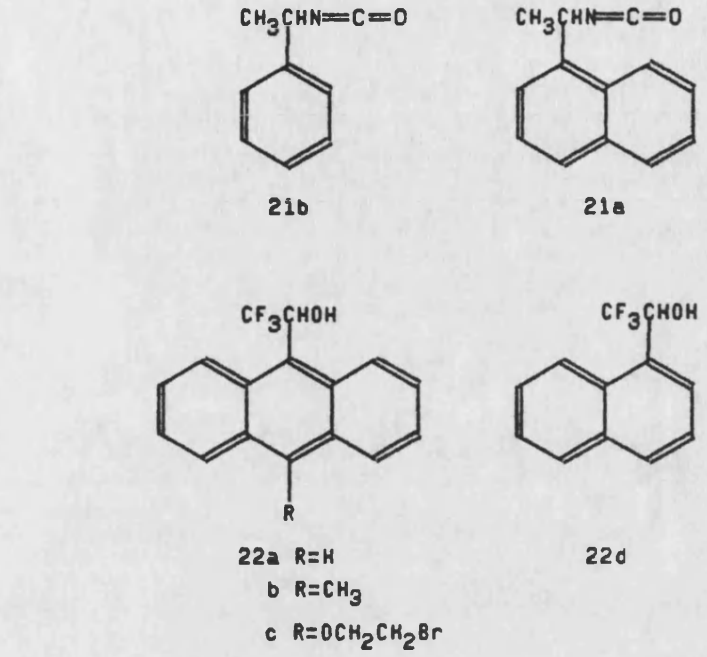
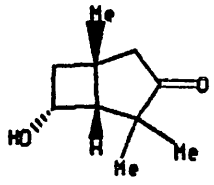


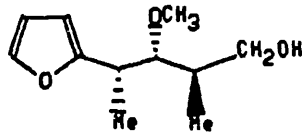
Fig 5.3. Automated repetitive chromatographic separation of the diastereomeric carbamates derived from 21a and 22d. The *R,R* diastereomer is the first of the two major bands.

Mori and Sasaki[31] resolved alcohol 23, a key intermediate in the synthesis of the pheromone lineatum, by chromatographically separating the diastereomeric

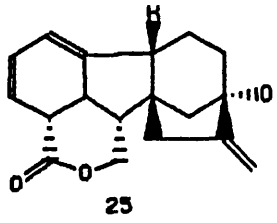
carbamates derived from CDA 21a. Schid and co-workers[32] utilised 21b to resolve alcohol 24, an intermediate in their monensin synthesis.



23



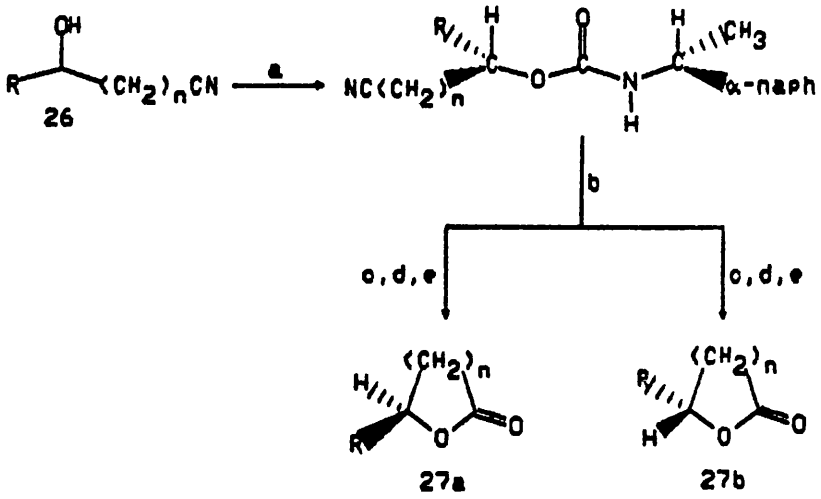
24



25

Diastereomeric carbamates derived from tertiary alcohols can also be separated chromatographically, as demonstrated by Corey and co-workers[33] in their synthesis of gibberellic acid, in which the resolution of alcohol 25 was accomplished using the carbamates derived from 21b.

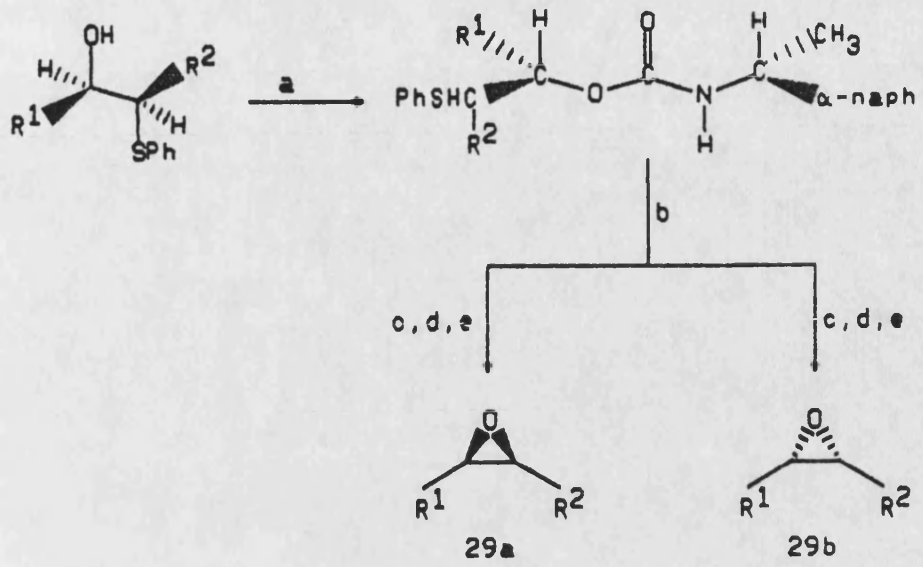
The advantage of indirect resolution is that the selectivity of the derivatisation and de-derivatisation reaction permits the presence of additional functionality. Diastereomeric carbamates derived from cyano alcohols 26 are valuable for the broad spectrum synthesis of optically active lactones.[15,34,35] These diastereomers are generally easy to separate (Table 5.2), and trichlorosilane cleavage and nitrile hydrolysis readily lead to optically active lactones 27 (Scheme 5.2). A number of optically active pheromones have been prepared.



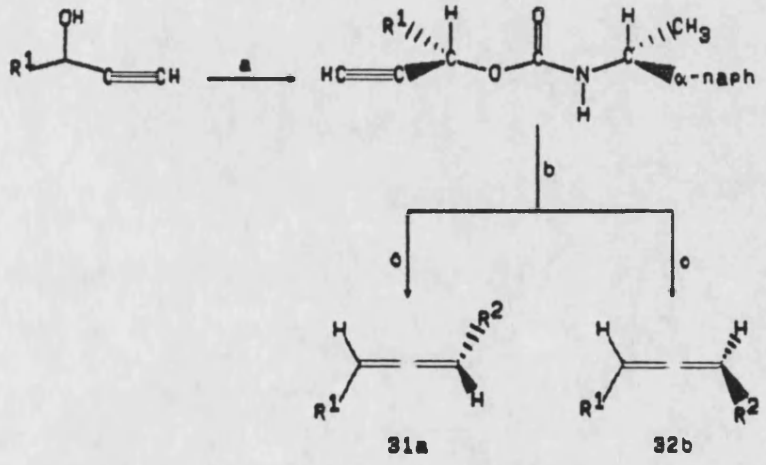
Scheme 5.2. a, 21a; b, chromatography; c, $HSiCl_3$; d, HO^-; e, lactonisation.

Optically active epoxides 29 can be obtained by carbamate resolution of β-hydroxysulphides 28, *S*-alkylation, and treatment with a base[14] (Scheme 5.3). This route was used in the synthesis of (+)-disparlone, the sex attractant of the gypsy moth.[36] Pirkle and Boeder[13] similarly resolved propargylic alcohols 30, some of which were used to prepare optically active allenes 31 (Scheme 5.4), including the sex pheromone of the male bean beetle.[37]

Below are given examples on chiral derivatisation of β-blockers of the 1-isopropyl-amino-2-propanol type. They are treated as a group as they have similar chemistry involved in derivatisation even though they may differ in terms of lipophilicity and spectral properties.



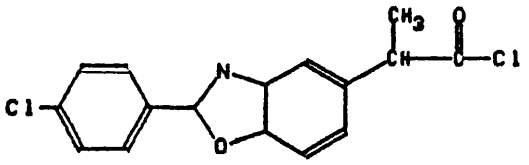
Scheme 5.3. a, 21a; b, chromatography; c, $HSiCl_3$; d, $MeOBF_4$; e, HO^-



Scheme 5.4. a, 21a; b, chromatography; c, R^2_2CuLi.

Benoxoprofen racemate was resolved by Weber *et al*[38] by preparative LC separation of the (*R*)-(+)-α-methylbenzylamide diastereomers. The *R* form was converted to (*R*)-benoxoprofen chloride **47** with an enantiomeric purity of

96.5%. Pflugmann et al[39] prepared crystalline derivatives of β-blockers, used as reference substances, by reaction in methylene chloride with anhydrous sodium carbonate.



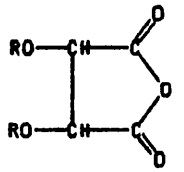
(47)

Metoprolol, oxprenolol and propranolol, extracted from urine, were derivatised with (R)-benoxoprofen chloride at room temperature overnight. Separation was performed on thin-layer silica plates and the pairs of diastereomers from the three β-blockers were adequately resolved. The absolute recovery of propranolol after extraction and derivatisation was 78%.

The symmetrical anhydrides of tert-butyloxycarbonyl-L-leucine, from commercial Boc-amino acids and phosgene, were prepared by Hermansson.[40] Propranolol was extracted from plasma and derivatised using the pure reagent. The reaction with alanine reagent was complete in ca 5min at room temperature, and the reaction with leucine was found to be 6X slower. Separation, after removal of the tert-butyloxycarbonyl group and extraction, was performed on a reverse phase LC system with the addition of

dimethyloctylamine in the mobile phase to reduce peak tailing. α Values of 1.3 and 1.7 were observed for the alanine and leucine derivatives respectively with detection levels in plasma as low as 1ng/cm^3.

Linder *et al*[41] approached the analytical and preparative resolution of the enantiomers of the β-blockers in a different manner by preparation of disubstituted (*R,R*) or (*S,S*)-tartaric acid anhydrides **45** an derivatisation with the β-blocker in an aprotic media. Very high separation factors were obtained on reverse phase systems for diastereomeric pairs from the different disubstituted

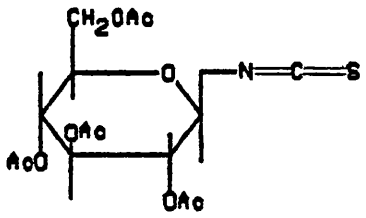


45

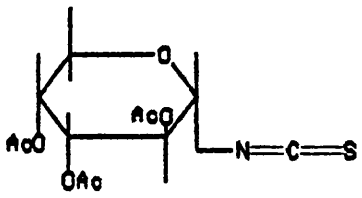
tartaric anhydrides. An intramolecular interaction involving the free carboxyl group of the tartaric acid residue and the free amino group of the β-blocker is given as the explanation for the separation observed. The enantiomeric purity of the reagent was reported as > 99.9%, while racemisation was < 0.1% for reaction with propranolol. Since the diastereomers are esters they are amenable to hydrolysis in order to recover the enantiomer. This, coupled with the high α values, makes derivatisation with substituted tartaric acid anhydrides a useful technique for determining the enantiomeric purity of β-

blockers.

Using the isothiocyanate reagents GITC 34 and AITC 35, Sedman and Gal[42] investigated the resolution of various β-blockers as thiourea derivatives. Using a reverse phase system, α values ranged from 1.21-1.28 for GITC derivatives and 1.09-1.22 for AITC derivatives. Christ and Walle[43] applied this method to a β-blocker metabolite, 4'-hydroxypropranolol sulphate, isolated from plasma, and achieved excellent resolution of the two enantiomers with detection levels down to 20ng of each enantiomer. Darmon and Thenot[44] reported a study on the use of napthylethyl isocyanate for the enantioselective determination of betaxolol in whole blood. Reverse phase chromatography gave base line separation (α=1.1) of the enantiomers.



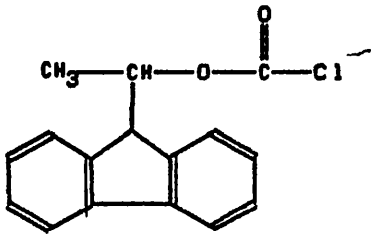
34 GITC



35 AITC

Einarsson et al[45] showed that β-blockers could be separated as carbamates after reaction with 1-(9-fluorenyl)ethyl chloroformate 36. Metoprolol was separated on a reverse phase system with an α value of 1.1.

The above examples are taken from just a few from the large and ever increasing number of papers illustrating



36 1-(9-fluorenyl)ethyl chloroformate

this method of resolution, and the widely different aspects of the methodology. Yet despite the ever increasing use of direct chromatographic methods, the indirect method will, at least, for the forseeable future, be continued to be used, due to increased knowledge of their rationale, efficiency and separation proces. A scan through the Journal of Chromatography between the years 1985-1989 serves to illustrate the still wide ranging use that this method of chiral recognition enjoys (Table 5.5 and 5.6).

Table 5.5. Selected applications from the Journal of Chromatography (1985-1989).

Compound	Reagent No;name	Appl[a]	Chrom[b]	Det[c]	Ref
AMINES					
Amphetamines	37 Naphthylsulphonyl-L-propyl chloride		M,R	UV	46
	38 Flunoxaprofen	plasma	M	UV	47
Tertairy amines	39 Phenylethylisocyanate		M	UV	48
Amino alcohols					
	40 OPA/Boc-L-cysteine	M	R	UV	
	41 α-Napthyl isocyanate		M	UV	49
	42 *o*-Phthalidaldehyde		R	FI	50
	43 (-)-Methylchloroformate		R	UV	51,52
Acebutolol/diacetolol	39 Phenylethylisocyanate		R	FI	53
Betaxolol	44 Naphthylethylisocyanate	blood	R	FI	44
β-blockers	45 Tartaric acid anhydrides		M	UV	54
Epinephrine	46 GITC	PP	R		55
Hydroxypropranolol	46 GITC	plasma	R	UV	56
Metoprolol	39 Phenylethylisocyanate	plasma	R	FI	39
Metoprolol/propranolol	47 Benoxaprofen chloride	urine	M	FI	39

Propranolol	45 Tartaric acid anhydrides		R	FI	57
	48 *tert*-Boc-Leu-anhydrides		R	FI	58

Free amino acids

	40 OPA/Boc-L-cysteine		R	FI	49
	49 OPA/*N*-acetyl-L-cysteine				59,49, 60
	50 OPA/*N*-acetyl-D-penicillamine		R	FI	49
	51 *N*,*N*-Diethyl-2,4-dinitro-fluoroaniline		R	UV	61
Aspartic acid	49 OPA/*N*-acetyl-L-cysteine		R	FI	62
Baclofen	49 OPA/*N*-acetyl-L-cysteine		R	FI	63

ALCOHOLS

Alkylglcerols	44 Naphthylethylisocyanate		N	UV	64
Deacetyltiazem	37 Naphthylsulphonyl-L-propyl chloride	ep	N	UV	46
1-Methyl-3-pyrrolidinol	52 Dibenzoyl tartaric acid	ep	R	UV	65

CARBOXYLIC ACIDS

	53 Naphthylethylamine	ac	N		66
2-Aryl propionic acids	54 L-Leucinamide	plasma	R	UV	67
	53 Naphthylethylamine	urine	N	UV	68

Caprofen	54 L-Leucinamide		R	FI	69
Flunoxaprofen	55 Phenylethylamine	BF	R	UV	70
Ketoprofen/indoprofen	54 L-Leucinamide	plasma	R	UV	71
Ibuprofen	56 Ethylchloroformate		N	UV	72
Pirprofen	55 Phenylethylamine			UV	73
N-Protected amino acids					
	57 Nitrobenzyltyrosine methyl ester		N	UV	74
THIOLS					
N-Acetyl penicillamine	58 OPA/Arginine		R	FI	75

a:- Appl, application; H, hydroylate; PF, pharamceutical formulation; ep, enatiomeric purity; ac, absolute configuration; BF, biological fluid; b:- Chrom, chromatographic mode; N, normal; R, reverse; c:- Det, detection mode; UV, absorbance detection; FI, fluresence detection.

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CHAPTER SIX.

DIRECT RESOLUTION.

6.1 General Introduction.

In recent years, greater attention has been devoted to devising methods for the direct resolution of enantiomers without prior derivatisation. These methods involve the use of a chiral stationary phase (CSP) that form transient diastereomeric complexes with solute enantiomers. The difference in stability between the complexes leads to a difference in retention time and when this process is repeated many times as the solute is eluted separation occurs. These methods avoid some of the drawbacks of the indirect method mentioned earlier and are relatively simple and less time consuming. CSPs can be used in both gas chromatography (GC) and high performance liquid chromatography (HPLC), but HPLC has the added advantage[1,2] that the enantiomers need not be volatile and they can be recovered with relative ease. Furthermore, GC requires more extensive clean-up procedures and the higher column temperatures can reduce the efficiency of the separations, or even cause racemisation. For these reasons the present trend is towards the use of CSPs whenever HPLC is considered for enantiomeric drug analysis.[3,4] Dappen *et al*[5] have discussed in detail the applications and limitation of commercially available CSPs for HPLC.

In this chapter, the various types of chiral stationary phase (CSP) are considered under two general types i) Empirical CSPs and ii) Designed CSPs. Ion-pair chromatography is considered separately, as the chiral selector is a mobile phase additive (CMPA), although strictly speaking it could be considered under the heading of empirical CSPs as separations are achieved as a result of practical experimentation and observation. In addition, computer assisted molecular modelling will also be briefly discussed.

6.2 Empirical Chiral Stationary Phases.

The concept of separating enantiomers by differential adsorption onto a CSP is not new. The expectation that the enantiomers of chiral dyes might be differentially adsorbed by naturally occurring enantiomerically pure materials was anticipated as early as 1923 by Ihrig and Porter.[6]

The initial discoveries of Senoh and Dalgleish have already been mentioned (CHAPTER 4, section 4.3), and attempts to use natural cellulose for enantiomer separation continue to be reported.[7] However, the chromatographic qualities of cellulose have been an impediment to their use as chiral supports, and attempts to use other polysaccharide such as Sephadex[8] and β-cyclodextrin[9-12] as CSPs have also been of limited success because of the poor mechanical properties of these saccharide and their unfavourable mass transfer characteristics. Slow adsorption

and desorption and/or slow diffusion through the polymeric network leads to band broadening with concurrent loss of resolution. Rather large α values are necessary if useful separation are to be afforded. In an effort to improve the chromatographic properties of cellulose, a number of chemical alterations have been attempted. Peracetylation of microcrystalline cellulose yields a triacetylcellulose (TAC) (CSP 3, Fig 6.1), which shows improved chromatographic behaviour and is capable of separating a wide range of enantiomers. First introduced by Hesse and Hagel,[13,14] microcrystalline cellulose is acetylated under

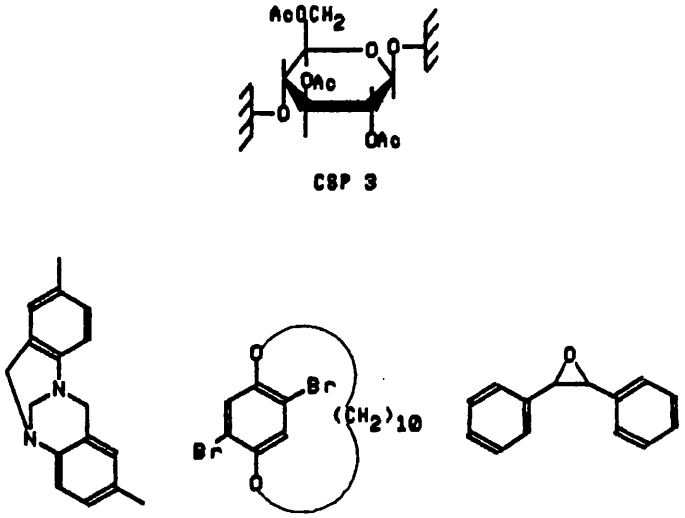
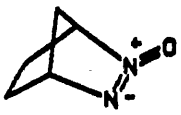


Fig 6.1. Monomer of triacetylcellulose, CSP 3, and some racemates separable on this CSP.

heterogeneous conditions in order to preserve the original crystal structure. It was found that solution and re-precipitin of TAC led to poor separation and in some cases reversal of elution orders.[15,16] Evidently, the tertiary

structure of TAC is of great importance in separations using this material. Several reviews of separation employing TAC and related materials are available,[17,18] and inexpensive TAC columns and packings are commercially available. Although many different enantiomeric separations have been achieved on these columns, little rationale for predicting separations is available. For this reason, the mechanism/s of chiral recognition has not yet been established, but an intercalation mechanism seem likely.[17-20] Nevertheless, the materials have been used in practice for a wide range of analytical and semi-preparative scale separations. TAC powder and various cellulose derivatives coated onto silica (triacetylcellulose, tribenzoate and triarylcarbamate) are now commercially available (Chiralcel materials; Diacel). Typical mobile phases include aqueous alcohol mixtures to hexane-isopropanol. Okamoto[21] has shown that the material obtained by bonding cellulose triarylcarbamate to silica had similar chromatographic efficiency to the coated material but with the advantage that more polar solvents could be used without dissolving the cellulose carbamate. Empirically it has been found that an aromatic or cyclohexyl ring close to the chiral centre is desirable but not a requirement (eg., the separation of the enantiomers of diazoxyalkane (74)[22]). Polar functionality on the substrate is not necessary, as a variety of helicenes and oxiranes have been separated on TAC.[23,24] Several of the other separations cited in Table 6.1 have been used for moderate amount (>100mg) of racemates.

Two proteins have been used as chiral selectors in LC and both have given rise to commercially available packing materials. Albumin is the principle plasma binding protein for weakly acidic drugs and α_1-acid glycoprotein (α_1-AGP),



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the major plasma binding protein for basic drugs. Stewart and Doherty[25] reported the chromatographic resolution of D,L-tryptophan using bovine serum albumin (BSA) bonded to agarose and thereby confirmed an earlier report using equilibrium dialysis, which suggested that L-tryptophan had an affinity for albumin one hundred times greater than that of D-tryptophan.[26] Following on such work from Allenmark and co-workers[27], a CSP with BSA covalently bonded to 7µm silica became commercially available (Resolvolsil; Macherey-Nagel). The BSA material can be used with aqueous buffer mobile phases for a wide range of analytical-scale separations[28], including sulphoxides, benzodiazepines and coumarins such as warfarin, as well as monitor the stereoselective microbial conversion of racemic substrates.[29]

As the protein-bound materials have such low sample capacity they have not been used for preparative-scale separations. The main interactions between BSA and the

solute enantiomers are hydrophobic and electrostatic interactions, although hydrogen bonding or charge transfer interactions could also contribute to chiral selection. In working with protein stationary phases, optimisation of chiral resolution can be achieved by varying the buffer pH or ionic strength and shorter retention times are obtained by adding an organic modifier such as propanol to the mobile phase. Albumin has also been used as a chiral mobile phase additive (CMPA) in reverse-phase chromatography with ODS-silica for the chiral resolution of carboxylic acids.[30] In 1983 Hermansson reported a silica-bonded α_1-AGP chiral material for LC and obtained useful resolution of a series of basic drugs. As with the BSA material, hydrophobic and electrostatic interactions are involved in the retention mechanism, along with hydrogen bonding[31]. The α_1-AGP/silica material is now commercially available (Enantiopak; LKB).

6.3 Designed Chiral Stationary Phases.

As the understanding of the mechanism whereby molecules interact with each other has increased, the design of CSPs which separate enantiomers by a pre-conceived and testable mechanism has become feasible. Such CSPs offer many advantages over the empirical CSPs discussed above. If the mechanism of enantio-differentiation is known (the "chiral recognition mechanism"), the order of elution for a given enantiomeric pair can be stipulated. Hence absolute configurations can

be assigned for many enantiomers, even though such assignments have not been made by more classical techniques.[32,33] Furthermore, a clear mechanistic understanding removes the empirical aspect of selecting a CSP to effect a given resolution. Finally, rationally designed CSPs are valuable tools for basic research into the mechanism by which chiral molecules interact, an important field of study applicable to many areas of chemistry and biochemistry.

At present, the design of CSPs for the chromatographic separation of enantiomers can be divided into three distinct categories. The first and most widely studied of these is ligand exchange chromatography (LEC). The theory and practice of LEC has been the subject of several reviews[34,35] and this subsection will attempt to update some of the developments that have occurred in the past seven years or so. The second type of designed CSP incorporates chiral cavities which selectively includes one enantiomer of a racemate. This category includes the imprinted polymers of Wulff,[36,37] the chiral crown ether phases designed by Cram and co-workers,[38] and the chiral polymers of Okamoto,[39] which are derived from triphenylmethyl-methacrylate. These CSPs warrant a separate discussion because they seem to behave in an understandable and predictable fashion. The last designed CSP to be discussed are the donor-acceptor or Pirkle type CSPs, which rely on weak interactions between the analyte and CSP to bring about separation. These weak interactions include hydrogen

bonding and dipolar, steric and van der Waals forces. Typically prepared by bonding a chiral precursor covalently to microparticulate silica, these CSPs resemble HPLC silica-bonded phases in chromatographic behaviour (high theoretical plates count, generally good kinetic behaviour), and are therefore the most easily adapted to routine use in research and analysis. These CSPs show exceptional scope and often afford such selectivity as to make facile preparative resolution of racemates. Since low-polarity volatile mobile phases are often used with these CSPs, they are especially well suited to preparative work. They have proved to be durable and long lived. The chief disadvantage of these CSPs is that certain type of analytes must be derivatised prior to analysis. In some instances, derivatisation may be undesirable, especially for preparative separations.

6.3.1 Chiral ligand exchange chromatography.

Chiral ligand exchange chromatography (LEC) was the first direct chiral resolution method that became easily accessible to the practising analyst. LEC has proved a powerful technique for the chiral resolution of un-derivatised amino acids. In the original system of Davankov and Rogozhin[39], L-proline was chemically bonded to a chloromethylated styrene-divinylbenzene co-polymer. Using an aqueous mobile phase containing Cu^{2+} ions, the racemic solute, D,L-proline, formed diastereomeric metal co-ordination complexes with the bonded amino acid and the

copper ions. As the ternary complexes formed with the individual solute enantiomers differed in energy, chromatographic resolution was achieved. Davankov has determined the conditions for optimising chiral resolutions with CSPs in LEC by varying the resin, metal ion, bonding chiral group and the nature, pH and ionic strength of the buffer.[34,41]

The poor mass transfer characteristics and long analysis times of the original polystyrene-based materials were improved in subsequent generations of chiral ligand exchange materials such as the silica-bonded material described by Linder.[42] The usefulness of the technique for both analytical and preparative scale separations has led to the appearance of several commercial CSPs for LEC. These utilise silica-bonded amino acids and are recommended for use with aqueous Cu^{2+} buffered mobile phases (Chiralpak, WE, WH, WM; Daicel: Nucleosil Chiral-1; Macherey-Nagel). The scope of chiral LEC was extended by Karger and co-workers[43,44] who were the first to use an achiral stationary phase (ODS-silica) along with an aqueous mobile phase containing the chiral selector. The latter was an optically active zinc or cadmium complex of a hydrophobic triamine and dansyl amino aids were well separated under alkaline conditions.

LEC was the first chromatographic method for the separation of enantiomers for which a testable mechanism was proposed. Based on the observed behaviour of the analyte enantiomers, it was proposed that the separations occur because of the difference in stability of the

complexes formed with the CSP, the stability difference being related to several factors, the most critical of which is the choice of the chiral portion of the stationery support. On non-polar supports, the *d*-amino acid is generally the most retained when *l*-proline is the chiral ligand. If the stationary support is polar (ie., capable of acting as a axial ligand for the metal ion in the diastereomeric complex), the *l*-amino acid is generally the most retained on the *l*-proline derived CSP.

Fig 6.2 illustrates the mechanism to account for the observed behaviour. Both amino acids involved in complex formation act as bidentate ligands, the amino and carboxylate groups being arranged alternately as equatorial ligands in a octahedral complex with the metal ion. The degree of steric interaction between the side chains of the amino acids, the presence or absence of axial ligands, and the nature of the second solvation sphere influences the sense of enantioselectivity.[45,46] On polar phases, such as CSP 4, in which the support provides an axial ligand for the metal ion, the *d,l* diastereomeric complex hinders the formation of the interaction, and hence is less stable. This polymer supported LEC CSP was designed specifically for optimum axial ligation of the copper ion.[47]

Conversely, the hydrophobicity of the sterically bulky alkyl groups in the *d,l*-complex shields the metal ion from the non-polar environment of the hydrophobic stationary support. Consequently, the *d,l* form is the more favoured and the *d*-enantiomer of an amino acid is generally more

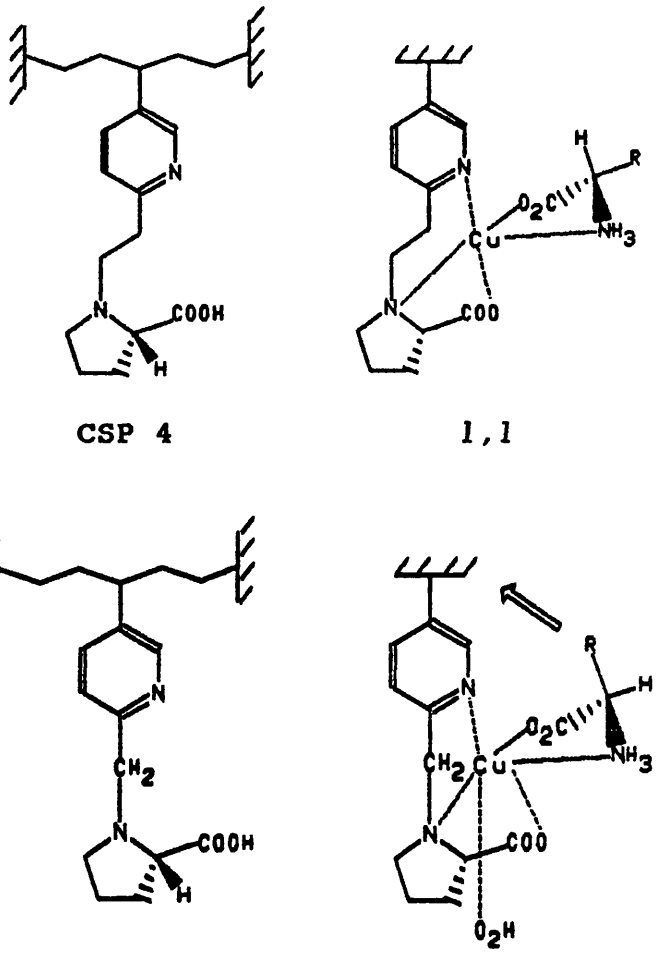


Fig 6.2. Control of enantiomer elution order in LEC by choice of achiral support.

retained on non-polar *l*-proline derived CSP 5.[48] Exceptions to these general trends occur on type CSP 5, when the amino acid to be resolved is tridentate in nature, such as ornithine, threonine or aspartic acid. Here, the *l*-forms are often the most retained.[49]

If a CMPA is used instead of a CSP for LEC, the situation becomes more complex. In this case, separations may result from a) intrinsic energetic differences between the diastereomeric complexes in solution (with the

complexes and the un-associated enantiomers having different partition co-efficients between the mobile and stationary phases, b) the diastereomeric complexes having different partition co-efficients, c) the CMPA concentrating in the stationary phase, converting it to a *de facto* CSP or d) a combination of all these effects. Finally, because at least three distinct entities are involved in the formation of each complex, entropy considerations are relatively more important in LEC than in normal phase chromatography, as noted previously (CHAPTER 4 section 4.4).

It is suitable to describe in more detail the advantage and disadvantages of using LEC as a routine means of enantiomer separation. For analytical purposes, LEC at present has no peer for monitoring the enantiomeric purity of un-derivatised amino acids. Since detection of un-derivatised amino acids can be difficult under the conditions of LEC, the amino acids may be derivatised prior to separation with a dansyl group or other fluorescent markers[50,51] or allowed to react with a derivatising agent such as phthalaldehyde after separation.[52] These techniques allow pico molar quantities of amino acids to be detected. LEC is compatible with biological samples because polar solvents are used and non-dedicated achiral columns are used. However, LEC can be applied only to selected bidentate analytes. While enantiomeric α-amino acids are readily separated by LEC as a rule, only poor separations have been obtained with β-amino acids.[53] Weinstein and

Grinberg[54] have separated the enantiomers of α-naphthyl-α-amino acids using a chiral mobile phase LEC system. Other workers have, however separated the enantiomers of catecholamines, amino alcohols, β-hydroxy-α-phenylethylamines, aliphatic and aromatic α-hydroxy-carboxylic acids.[55-58] Although LEC has been successfully utilised for preparative purposes,[59] difficulties with removal of polar mobile phases, recovery of the CMPA (should one be used), and isolation and purification of the now separated enantiomers may be encountered. One approach of the removal of the metal ion is to pre-charge the CSP with the metal ion[47] and pass the effluent through a scavenger column to remove traces of metal ion which may elute.[60] An additional chromatographic disadvantage of LEC is the generally low rates of desorption, broad bands and long retention times are often seen.[61] Many attempts have been made to improve LEC kinetics by addition of monodentate ligands such as ammonia to the mobile phase to increase exchange rates.[62] LEC is generally conducted at elevated temperatures to minimise the effect of slow kinetics.[61]

6.3.2 Chiral stationary phases with chiral cavities.

Cram and co-workers[63,64] after extensive studies of the water-chloroform partitioning of diastereomeric complexes formed between chiral crown ethers and the enantiomers of a variety of chiral ammonium salts, prepared a number of silica- and polymer-supported crown ethers CSPs. These were

used for the separation of enantiomers of chiral amines, amino acids and amino esters all as their ammonium salts.[65] From solution studies the complex most favoured was found to have the least steric hinderance between the naphthyl rings of the crown ether and the R-group of the amino acid, while still maintaining strong hydrogen bonding interactions between the ammonium proton and the ether oxygens (Fig 6.3). Although elegant in the extreme, the difficulty of preparing these CSPs and the relatively poor peak shapes they afford have precluded any general attempt at usage. However, the work has stimulated considerable interest in other types of chiral crown ethers.

The cyclodextrins are a group of cyclic oligosaccharides which have found wide application in chiral LC. α, β and γ cyclodextrins, containing 6, 7 or 8 D-glucose units respectively, each possessing a chiral cavity proportional to the number of glucose units present (eg., 8Å for β-cyclodextrin) and the materials are capable of forming inclusion complexes with a variety of racemate. The inclusion complex is formed between the hydrophobic groups of the guest molecule and the relatively hydrophobic chiral cavity of the cyclodextrin and involves various hydrophobic and hydrogen bonding forces. These CD complexation processes are highly stereoselective, and can be considered as the method of choice for the resolution of various isomers namely:- structural; geometrical; diastereomeric and enantiomeric isomers. Initially the materials were used in water soluble form to generate diastereomeric inclusion

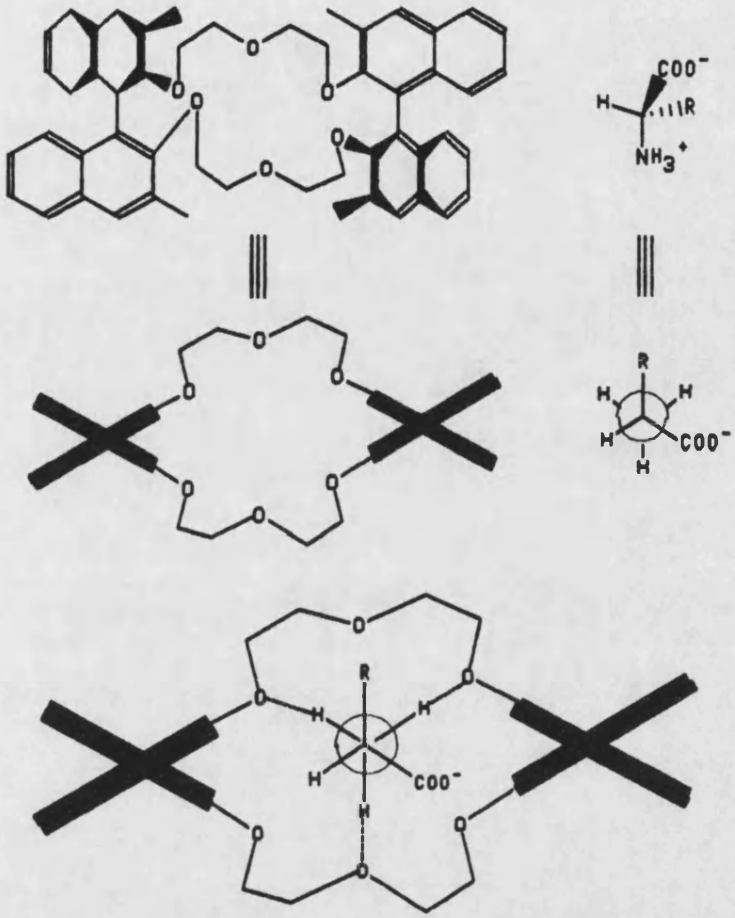
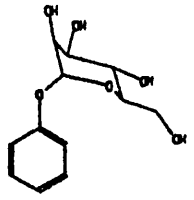


Fig 6.3. Most favoured diastereomeric host-guest complex between an a amino acid and a chiral crown ether.

complexes which could be separated by fractional crystallisation to give enantiomer enrichment. Modern commercially available materials for HPLC include cyclodextrin and acteylcyclodextrin bonded to 5µm spherical silica (Cyclobond range; Astec). These materials are used in reverse-phase mode with an aqueous alcohol mobile phase[66] and have been employed successfully in chiral separations of prostaglandins, sulphoxides and vitamins. Partial chiral resolution of mandelic acid was also achieved using β-

cyclodextrin as a CMPA in conjunction with an achiral reverse-phase column.

Wulff and co-workers[67] approached in a different way, the construction of chiral cavities in a stationary support. They pioneered the use of chiral templates to link chiral cavities into a polymeric framework. Typically one enantiomer of the template molecule is bound by an easily cleaved linkage, such as a Schiff base or boronic ester, to a monomeric unit, which is allowed to polymerise with a second monomer having cross-linking capability. Subsequent chromatography of the racemic template compound often gives separation of the enantiomers. A typical example is the separation of the enantiomers of nitrophenyl manopyranoside (75) on a chiral polymer derived from methyl methacrylate, ethylenedimethacrylate and the 4-styrenylborate ester of α-d-4-nitrophenylmanopyranoside. A separation factor of 1.4 was obtained, although baseline separation was not seen.[68]



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As was expected, a high degree of cross-linking is necessary for the separation of enantiomers to occur on these CSPs, otherwise the large "holes" produced are effectively achiral. Moreover, swelling of the polymer by solvents distorts the chiral holes, and diminishes

separation.[64] Such imprinted CSPs may eventually prove useful for specific applications such as repeated large-scale resolution of a particular racemate. They are also of theoretical interest as models for synthetic enzymes, since the stereo- and regio-chemical course of a reaction may be influenced by the presence of a properly templated polymer.[36,37]

CSPs in which the chiral selector is a synthetic polymer have only recently become available, although their conception is not recent. The origins of these material lies in the theory proposed by Pauling[27] which accounts for the natural formation of antibodies specific for a given antigen. Pauling proposed that the antigen protein adopts a tertiary structure complimentary to that of the antigen template. This idea was later applied to the preparation of silica.[70,71] Silica prepared in the presence of methyl orange or related azo dyes was shown to adsorb specifically the given template molecule and in 1949 Dickie[70] mentioned that he was investigating "the possibility that optical isomers can be separated with such adsorbents". Later Wulff and Sarahan[72] prepared a series of enzyme-analogue polymers for chiral LC. Wulff's method involved synthesising highly cross-linked co-polymers which incorporated chiral carbohydrate template molecules. Subsequent removal of the template by hydrolysis yielded polymers with chiral cavities which were used to resolve racemates of the template molecules.

The polymers have also been bonded to silica,[74] but the

chromatographic efficiency of such materials is fairly poor. Okamoto and co-workers[74,75] polymerised triphenylmethacrylate in the presence of a chiral anionic initiator and obtained a polymer, the chirality of which was due solely to its helicity. Modern materials for chiral LC in which the polymer is bonded to silica are commercially available (Chiralpak OT and OP; Diacel). In the OP material, the phenyl groups are replaced by pyrazine. Recently the chiral template approach was applied to another type of CSP. Aubel and Rogers[76] reported initial results regarding the possibility of re-orienting the tertiary structure of silica-bonded BSA in the presence of a single enantiomer.

In a very recent advance, synthetic polymer CSPs have been prepared commercially using optically active monomers as building blocks.[77] Development of these materials has led to the commercial exploitation of a silica-bonded chiral polymer prepared from N-acryloyl-(*S*)-phenylalanine ethyl ester (Chiraspher; E. Merck).

As chiral recognition on CSPs with chiral cavities is dependant on the formation of inclusion complexes, the chromatographic efficiency afforded by such systems is invariably worse than that of standard HPLC columns. This is a limitation for analytical applications to enantiomers which afford but modest level of chiral recognition or for samples containing a number of components.

6.3.3 Donor-acceptor type chiral stationary phases.

CSPs based on CSP-analyte interactions weaker than ionic interactions encountered in LEC will, for the purposes of this discussion, be called donor-acceptor CSPs. In general (but not exclusively), relatively non-polar mobile phases are used for the CSPs and chromatographic conditions and parameters are similar to those of typical bonded phases. Although a variety of CSPs of this type have been prepared in the past ten years, the principles behind most of them are similar. Many of these CSPs contain aromatic or π functionality, which may take part in a van der Waals (π-π), donor-acceptor or dipole-stacking complex with the analyte. The importance of aromatic groups for the success of donor-acceptor CSPs lies in the nature of the donor-acceptor interaction between the species. The relative energy of the π-orbitals (HOMO and LUMO) in aromatic systems, is determined largely by the nature of the substituents on the aromatic system. Electron-withdrawing groups such as NO_2 tend to lower the orbital energies (HOMO), making nitro-substituted aromatics good π-electron acceptors. Electron-donating groups such as amines and hydroxyl groups, raise the relative energies of the π-orbitals (LUMO) making such aromatic systems good π-electron donors. The donor-acceptor interaction involves the approach of the frontier π-orbitals of the aromatic systems in a parallel fashion. For the most effective interaction, the highest occupied molecular orbital (HOMO) of the π-donor species is isoenergetic with the lowest

unoccupied molecular orbital (LUMO) of the π-acceptor system. As the frontier orbitals approach each other, the LUMO is raised in energy while the HOMO is lowered, as called for by the perturbation theory, and the lowering in energy of the HOMO determines the strength of the interaction (Fig 6.4).[78] Since the orbitals must be non-orthogonal to interact, the rings interact most strongly

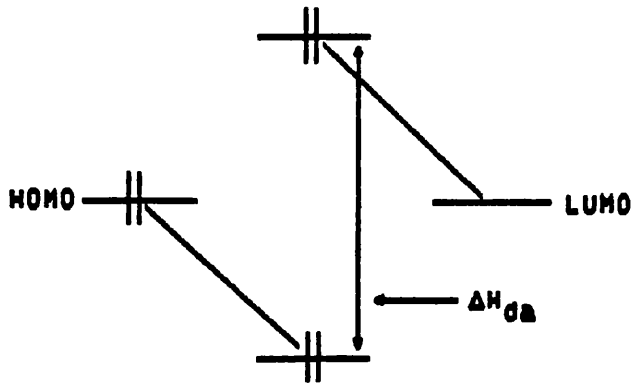


Fig 6.4. Energy diagram of π-donor-acceptor complex formation. On close approach, the LUMO is raised in energy, while the HOMO is lowered by an energy $\frac{1}{2}\Delta H_{da}$, resulting in a net gain in energy of ΔH_{da} for a fully occupied HOMO.

when they are parallel. Thus the strength of the donor-acceptor interaction between the aromatic moieties of the two molecules is dependant on the relative position of these groups. The face-to-face nature of these donor-acceptor interactions places restrictions on the relative motion of the aryl rings. Such interactions constitute more than a single point interaction. When the aryl rings show

conformational preference relative to the remainder of the chiral molecule, the restrictive nature of the donor-acceptor interaction may prove invaluable for chiral recognition. It is no accident that CSPs utilising these donor-acceptor interactions are among the most effective known.

An interesting consequence of strong π-donor-acceptor interactions is the appearance of a charge-transfer band in the visible spectrum of such complexes. The enantiomer incorporated into the more stable adsorbates will often give a much more deeply coloured complex with a chiral π-acceptor than does it antipode, and column separations can often be monitored by the progress of two coloured bands.[79]

Hydrogen bonding, dipole-dipole and steric interaction effects are also frequently present in these CSPs, and although not necessarily directly responsible for high enantioselectivities[80] are nevertheless frequent contributors to the observed separations.

The precursors used to prepare donor-acceptor CSPs are often readily available enantiomerically pure materials, such as amino acids or α-arylalkyl amine and carbinols, which are resolved either chromatographically or by classical methods. Appropriate introduction of functionality is followed by attachment of the precursor to a solid support, usually silica gel. Since the design of such stationary phases is almost invariably based on 1:1 interactions between the CSP and analyte, the mechanism involved can often be deduced.[80]

Some early successes in the design of donor-acceptor CSP were obtained by Mikes and Boshart.[81,82] Using chiral 1,1'-binaphthylphosphate esters and nitroflurenyl-derivatives of hydroxypropionic acid bound to silica supports, these workers reported successful separation of the enantiomers of a number of helicenes. Despite low separation factors, the high efficiencies of these stationary phases allowed complete separation of enantiomers ($\alpha>1$), indicating that for many analytical and preparative tasks, donor-acceptor silica bonded CSPs were the method of choice.

In his first CSP, Pirkle[83] bonded a chiral anthryl alcohol to silica and used it to resolve a wide range of racemates (sulphoxides, lactones and derivatives of amines, amino acids and alcohols). An important interaction in the chiral selection process was the π-π interaction between the anthryl π-base substituent and a π-acid group on the solute enantiomer (eg., a nitroaromatic group), while hydrogen bonding between the stationary phase and the enantiomeric solute was also important. Pirkle's detailed understanding of the interaction between the CSP and an enantiomeric solute led to the possibility of predicting the elution order of individual enantiomers. In generalised form the process of chiral selection is illustrated in Fig 6.5. In Fig 6.5a, the chiral selector in the stationary phase forms three interactions with one enantiomer whereas only two interactions are possible with its mirror image (Fig 6.5b). The latter is therefore less strongly bound by

the CSP and will elute first. Pirkle utilised the reciprocity of the chiral selection process to prepare a second generation of CSPs. His original anthryl alcohol CSP gave good resolution of dinitrobenzoylphenylglycine enantiomers and hence dinitrobenzoylphenylglycine was bonded to the surface of silica.[84] The resulting CSPs possessed such wide versatility for chiral resolution that

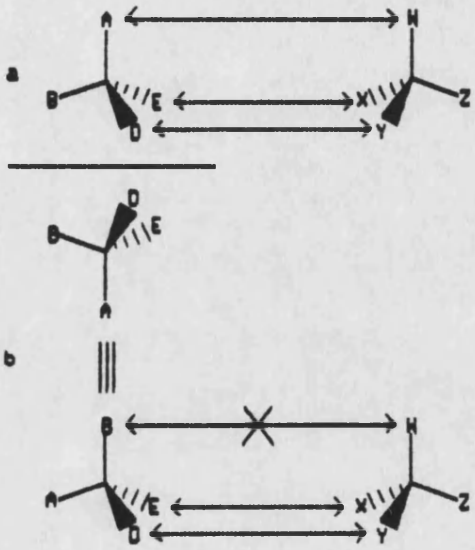


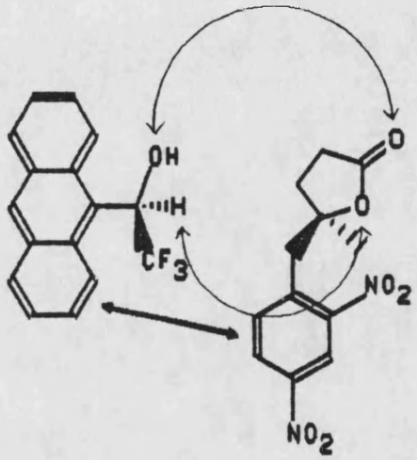
Fig 6.5. Generalised representation of the tree-point rule.

two materials with *N*-(3,5-dinitrobenzoyl)phenylglycine ionically or covalently bonded to silica are commercially available (J.T. Baker; Regis). The material are used for normal-phase chromatography, for example with hexane-isopropanol mobile phases. An intrinsic advantage of having the chiral selector in the stationary phase is that only small quantities of the chiral selector are required, in

contrast to diastereomer formation or methods in which the chiral selector is a component of the mobile phase. Further more, as the Pirkle CSPs are well suited to preparative scale separations[85], the presence of a bonded chiral selector is advantageous because in cases where the chiral selector is a component of the mobile phase, it must be subsequently separated from the resolved enantiomer fraction.

Pirkle and co-workers[86] performed a series of solution magnetic resonance studies of chemical shift non-equivalence between enantiomers induced by the presence of a chiral reagent known as chiral solvating agent (CSA). It was shown that chemical shift non-equivalence could be induced between the enantiomers of a variety of racemates by complexation with single enantiomers of a series of trifluoromethyaryl carbinols.[86] The non-equivalence was ascribed to the formation of diastereomeric complexes of the enantiomers of the racemate and the carbinol. Often only two points of direct interaction between the CSA and analyte were invoked to account for the observed chemical shift non-equivalence, and the essential third "interaction" being a perturbation of the magnetic environment. It was recognised that often more than two interactions were involved,[87] and that in such instances magnetic non-equivalence might be indicative of energetic differences between the diastereomeric complexes. Such differences are not essential to the chemical shift non-equivalence, although they may contribute to this non-

equivalence if present. Chromatography of the π-acceptor substituted lactone 76 on silica gel using the (*R*)-trifluoromethyl carbinol 77 as the CMPA resulted in enrichment of (*R*)-76 in the first eluted fraction (Fig 6.6). The enrichment resulted from the preferential solvation of the (*R*)-76 by the CMPA, causing it to be less retained. Preferential solvation results from the three-point rule interaction between the (*R*)-76 and the (*R*)-77 (Fig 7.5).[88] Interactions include a hydrogen bond between

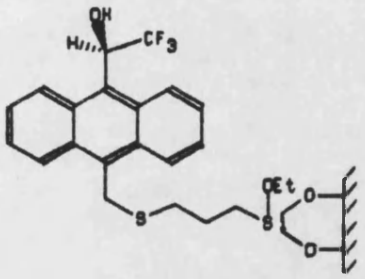


77 76

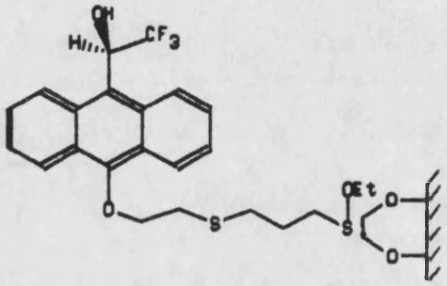
Fig 6.6. Three-point interaction between lactone 76 and chiral solvating agent 77 in the favoured diastereomeric complex.

the hydroxyl proton on 77 and the carbonyl oxygen of 76, a second weaker hydrogen bond between the carbonyl hydrogen of 77 and the lactone ring oxygen of 76, and a π-donor-acceptor complex between the aromatic rings. Covalent linkage of enantiomerically pure 2,2,2-trifluoro-1-[9-(10-

bromomethyl)anthryl]ethanol to a silica support through a sulphide linkage resulted in CSP 6 capable of separating the enantiomers of a large number of π-acceptor substituted amine, amino acids and sulphoxides.[83] Increasing the π-donor nature of the anthryl ring (CSP 7), substituting bromomethoxy for bromomethyl, improves the performance, indicating that the π-π interactions are indeed important in the separation of these enantiomers.[89] The high degree of



CSP 6

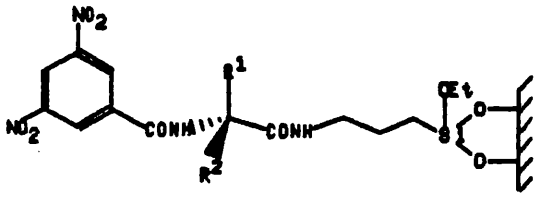


CSP 7

separation displayed on CSP 6 and 7 by the enantiomers of N-(3,5-dinitrobenzoyl) amino acids derivatives indicated that these analytes might themselves be suitable candidates for incorporation into CSPs which could separate the enantiomers of π-donor substituted species. This inverse relationship between CSP and analyte has been called the reciprocality concept, and within limits, is a useful guide to the development of CSPs.[90] That *N*-(3,5-dinitrobenzoyl) amino acids were useful CSP precursors was demonstrated when these π-acceptor CSPs, particularly those derived from (*R*)-phenylglycine (CSP 8) and (*S*)-leucine (CSP 9), found rapid acceptance for the chromatographic separation of the

enantiomers of a myriad of π-donor substituted amino alcohols, amino acids, sulphoxides and other compounds (Fig 6.7).[91]

Further application of the concept of reciprocality, prompted the generation of a third generation of donor-acceptor CSPs, based on compounds which incorporate a good



CSP 8 R^1=Ph R^2=H

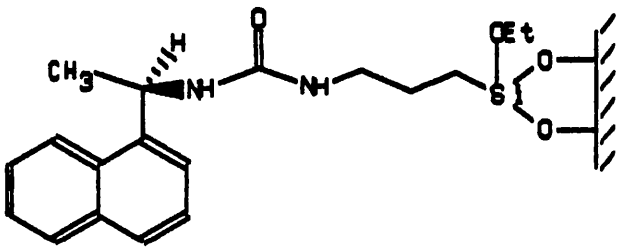
CSP 9 R^1=H R^2=*i*-Bu

Fig 6.7. π-acceptor CSPs derived from *N*-(3,5-dinitrobenzoyl)-derivatives of phenylglycine and leucine.

π-donor, and which show a high degree of separation on the *N*-(3,5-dinitrobenzoyl) CSP 8 and 9. Racemates separable on these "third generation" CSPs must, in general, be derivatised in order to incorporate the functionality necessary for separation.[92-94] Derivatisation usually incorporates a electron-deficient π-system and is often accomplished by acylation of amino and hydroxyl groups with 3,5-dinitrobenzoyl chloride or 3,5-dinitrobenzoyl-phenylisocyante. Carboxylic acids have been resolved as their dinitroanilide derivatives.[94]

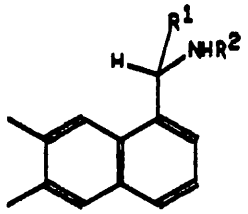
Many of these π-donor CSPs have been derived from 1-aryl-1-amino alkanes. Oi and co-workers[92,93,95,96] have

demonstrated the usefulness of CSP 10, prepared from enantiomerically pure 1-(1-naphthyl)-ethylisocyante and aminopropyl-derivatised silica gel, for the separation of the enantiomers of *N*- and *O*-dinitrobenzoyl and dinitroanalido-derivatised amines, alcohols and related compounds. Pirkle and Hyun,[97] using a series of related CSPs, demonstrated that alkyl substitution of the aromatic

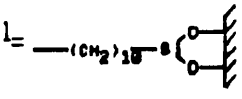


CSP 10

functionality of the homologs of 1-(1-naphthyl)-1-amino alkanes, bonded to silica either by am amide linkage (CSP 11) or through the alkyl substituent (CSP 12), increase the



CSP 11 R^1=i-Pr R^2= 

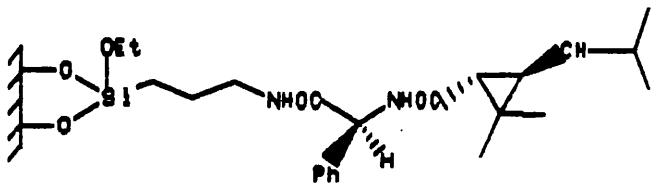
CSP 12 R^1=  R^2=Ac

π-donor nature of the CSP, and consequently the extent of separation seen for a wide variety of racemates. They further demonstrated that the mode of attachment of the chiral α-arylalkyl amine to the silica support was of great significance for determining the extent and sense of chiral recognition, as more than one mechanism for chiral recognition is often operative on these types of CSPs. In the event of multiple chiral recognition mechanisms, these may differ in degree and sense of selectivity. It was noted that inversion of the elution order of enantiomers in a homologous series was often seen as the alkyl chain length of a given substituent increased. For example, the homologous series of *N*-(3,5-dinitrobenzoyl)-1-aryl-1-amino alkanes show, on CSP 12, an inversion of elution order from (*R*), most retained, to (*S*), least retained, as the chain length of the alkane increases. This behaviour has been explained in terms of competing chiral recognition processes of opposite enantioselectivity, the contribution from each being influenced by the length of the alkyl chain.[98-100]

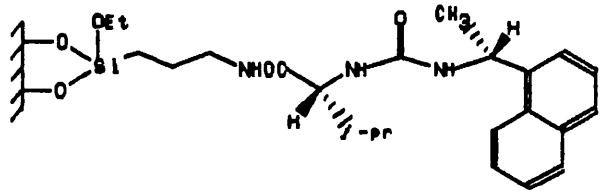
A number of donor-acceptor CSPs not incorporating strong π-donor or π-acceptor groups have evolved, based on *N*-acyl amino acids. CSPs developed by Hara and Oi, are related to CSPs developed for GC,[101] and are capable of separating the enantiomers of *N*-acyl amino esters.[102-104] These depend on hydrogen bonding and/or amide dipole interactions for their effectiveness. Either the dual hydrogen bonds or dipole-stacks may be considered analogous

to a π-donor-acceptor interaction in that both are also two-point interactions, and therefore conformationally restrictive. The most effective phases of this type are those which are prepared from the amino acid *N*-formamides. Increasing the steric bulk of the amide substituent tends to decrease the effectiveness of the CSP.[105]

Several hybrids CSPs incorporating both π-donor and an amide functionality, often with multiple chiral centres, have been developed by Oi and co-workers (CSP 13 and 14).[93,106] These are prepared from acylated amino acids and chrysanthemic acid bonded covalently to silica through a variety of linkages, and can be used to separate a wide variety of racemates, but the relative complexity of these phases makes it difficult to establish a coherent chiral recognition model for them. Pirkle has studied π-donor CSPs



CSP 13



CSP 14

which may be prepared simply and directly from enantiomerically pure starting materials, such as N-aroylamino acids,[107,108] and show a very high degree of enantiomeric separation on CSP 8 and 9. Chromatographic studies of the relationship between the structure of the *N*-aryl amino acids and their enantiomeric separability, allowed optimisation of the designs of these π-donor CSPs.[32] As the δG values, calculated from the separation factors observed for the diastereomeric adsorbates of *N*-aryl amino acids derivatives on CSP 8 and 9, are quite large, (as large as 6.7kJ mol^{-1} in some cases), spectroscopic studies of the interactions of these analytes with soluble analogs of the CSPs was undertaken. Significant ultra-violet (UV) and NMR spectral differences were noted for the diastereomeric complexes. Such data, combined with observed <u>intermolecular</u> nuclear Overhauser effects, allowed for accurate delineation of the interactions responsible for chiral recognition in solution of *N*-aryl amino esters and the soluble analogs of CSP 9.[109] These interactions (Fig 6.8) involve π-π interaction and two hydrogen bonds. Steric interactions are implicit in controlling the conformational preferences of the enantiomers. The complex (Fig 6.8) represents the structure of the more stable diastereomeric complex formed by interaction of the enantiomers of *N*-aryl amino esters and those of *N*-(3,5-dinitrobenzoyl)-amino acid derivatives. This finding may be generalised to account for the enantiomeric elution of a variety of analytes on CSPs derived from *N*-aryl amino esters.[80] This mechanistic

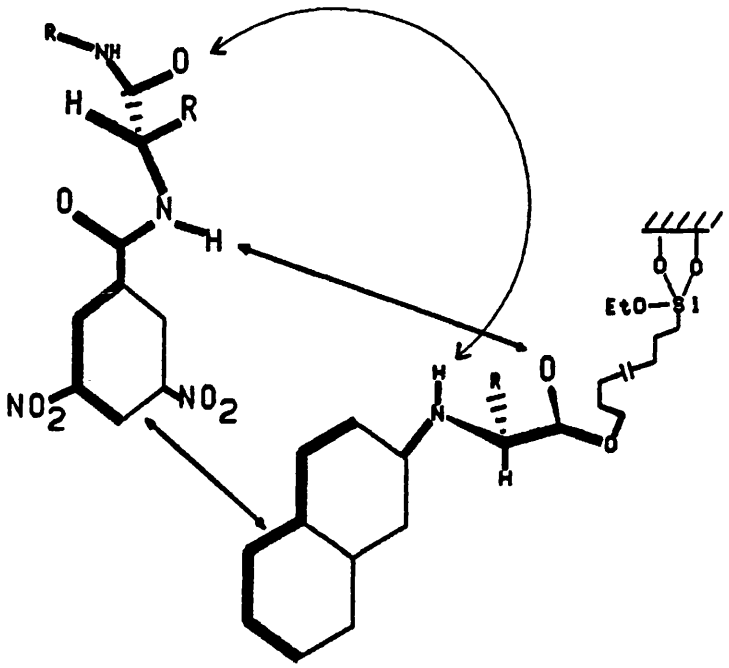
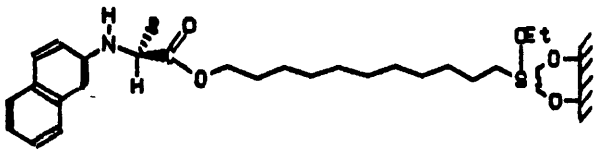


Fig 6.8. More favoured diastereomeric complex between an *N*-(3,5-dinitrobenzoyl)-α-amino amide and an *N*-(2-napthyl)-α-amino ester.

uniformity permits assignment of absolute configuration to analyte enantiomers based simply on their elution order.

The most generally useful CSPs yet derived from *N*-aryl amino esters have been prepared from alanine (CSP 15) and valine (CSP 16). Both of these separate the enantiomers of a wide variety of *N*-(3,5-dinitrobenzoyl)- and *N*-(3,5-dinitroanilido)-derivatives of amines and amino-substituted compounds. On the other hand 3,5-dinitrobenzoyl esters of chiral alcohols show little enantiomeric separation on CSP 15 and 16 because they lack the acidic proton necessary for chiral recognition (Fig 6.8). Therefore, chiral alcohols are usually best derivatised with 3,5-dinitrophenyl-



CSP 15 R=Me

CSP 16 R=i-Pr

isocyanate in order to achieve usable separations, the resulting dinitrophenyl carbamates containing the almost indispensable acidic N-H proton.

CSP 15 and 16 are somewhat limited in scope, generally requiring that the chiral centre also be the point of hetero-substitution and derivatisation. CSP 11 and 12 have been found to separate the enantiomers of compounds in which the chirality was one or two carbons removed from the derivatised substituent. CSP 15 and 16, however, generally give more rapid turnaround times than CSP 11 and 12, require less polar mobile phases and are more predictable in their behaviour. The lower cost and ease of preparation of CSP 15 and 16 (as opposed to CSP 11 and 12) have also allowed their rapid commercialisation (Regis). Pirkle and co-workers have also prepared several preparative scale MPLC columns packed with CSP 15 and 16 bonded to larger irregular silica particles. Fig 6.9 shows the chromatogram of the separation of 0.5g of ethyl α-hydroxyisovalerate as the dinitrophenyl carbamate derivative on 300g of CSP 16 bonded to 40μm Brinkman silica gel. These MPLC columns have allowed the preparative separation of the enantiomers of a

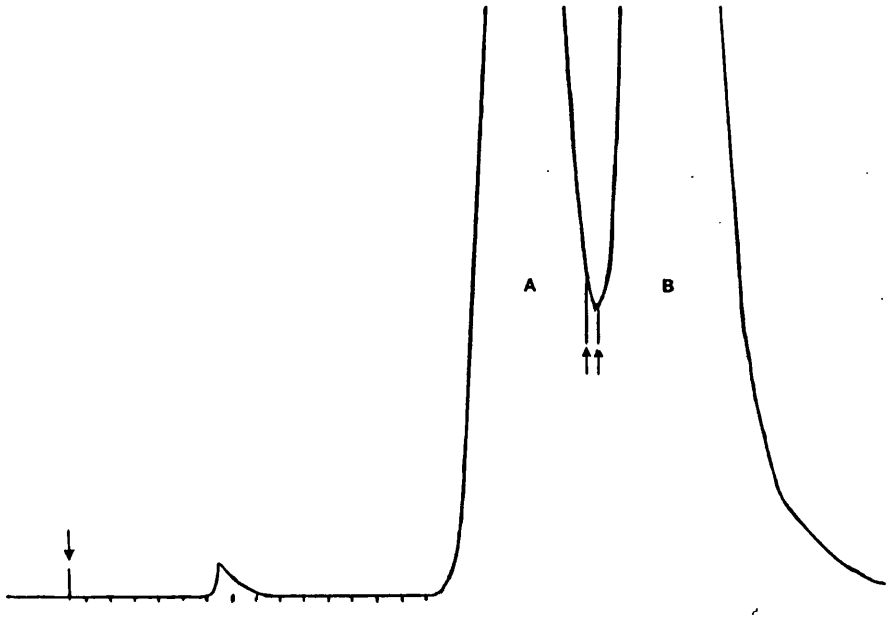


Fig 6.9. Preparative separation of methyl α-hydroxy-isovalerate as the O-(3,5-dinitrophenyl)carbamate using CSP (*R*)-16 as chiral stationary phase.

number of chiral alcohols as their 3,5-dinitrophenyl carbamates, including 1-phenylethanol, 1-tetralol and a number of 1,2-alkandiols.[79] The enantiomerically pure free alcohols were then recovered by cleavage of the carbamate with $HSiCl_3/Et_3N$.[112]

6.4 Chiral Mobile Phase Additives.

A chiral counter-ion dissolved in the mobile phase can be used to separate enantiomers of acids and amines. The basis for such resolutions is the formation of diastereomeric ion-pairs with different stabilities or distribution properties between the mobile phase and the stationary phase.

Pettersson and Schill[111] separated enantiomeric 1-aryloxy-3-isopropyl-amino-2-propanol derivatives using (+)-10-camphor-sulphonate as the counter ion in the mobile phase. The solutes, such as alprenolol, metoprolol, and propranolol, were resolved using silica gel columns with eluting solvents that contained as low a proportion of polar solvent, such as alcohol, as possible. Stereoselective association was believed to occur via diastereomeric ion-pair formation between solutes and the chiral (+)-10-camphorsulphonate counter ion. Such ion-pairs have structural differences substantial enough to distribute themselves differently between the organic mobile phase and the stationary adsorbent. The assumption that interaction at three-points adjacent to the chiral carbon atom is necessary for stereoselectivity was believed to be valid in this work. The necessary interactions in the ion-pair stereoselection process seem to be i) electrostatic attraction, ii) hydrogen bonding between the hydroxyl of the solute β-chain and the oxo group of the camphorsulphonate, and iii) hydrophobic interaction between the ring systems.

The influence of hydrogen bonding agents in the mobile phase was found to be detrimental. In no cases could resolution be obtained if water was even a low concentration mobile phase component. Lipophilic alcohols of different structure, such as 1-pentanol and isopropanol, gave about the same selectivity, and hydrogen-donating agents gave slightly higher separation factors, but less

symmetrical peaks (Table 6.2). Table 6.3 illustrates the structural effects of the sample on the separation factor. Oxprenolol enantiomers are unresolved probably due to the presence of the 2-alkoxy group at such a position in the ring that internal hydrogen bonding with the side chain hydroxyl was possible. Terolidine, which lacked a hydroxyl group, and hyosyamine, with its hydroxyl substituent too far removed from the amino group, were also unresolved.

Table 6.2. Influence of polar components in the mobile phase.

Polar solvent	content (%)	k'(+)	α	Asf[a]
Pentanol	0.5	20.3	1.08	1.5
	1.0	10.8	1.06	1.1
	5.0	2.0	1.02	1.1
Isopropanol	0.5	13.5	1.07	2.6
Acetonitrile	1.0	25.1	1.09	2.2
Tetrahydrofuran	1.0	11.9	1.10	3.7
Ethyl acetate	1.0	29.0	1.10	3.6

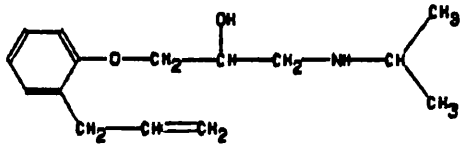
a:- Asf, back part of the peak/front part of the peak.

Use of (+)-3-bromo-10-camphorsulphonate counter ions caused total stereoselectivity loss, probably due to steric interference which prevented good ion-pair formation.

Table 6.3. Separation of enantiomers.

Enantiomer	k'(+)	k'(-)	α
Alprenolol	24.9	27.4	1.10
Metropolol	34.9	38.7	1.11
Oxprenolol	15.0	15.0	1.00
Propranolol	40.9	46.0	1.12
Terodiline	4.9	4.9	1.00
Hyoscyamine	7.3	7.3	1.00

Pettersson and Schill[112] extended their work with chiral counter ions to next study separation of 10-camphorsulphonic acid on a hydrophillic adsorbent with (+)-alprenolol (78) as the chiral counter ion in the organic mobile phase. Pettersson and No[113] presented extensive results of a study of resolution of enantiomeric carboxylic



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and sulphonic acids using the cinchona alkaloids, quinine (79), cinchonidine or quinidine (80) as the chiral counter ion (Fig 6.10). A further benefit of the study was the demonstration that detection could be improved, with high UV-adsorbing counter ion species. Even non-UV-adsorbing acids and anions could yield a UV response and the detection at low levels.[114]

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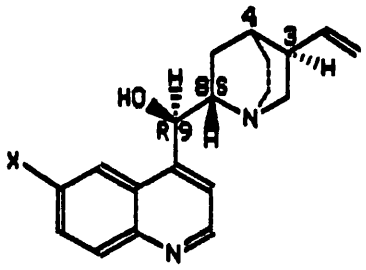

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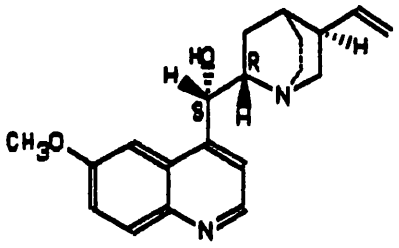


Fig 6.10. 79 Quinine X=OCH_3, [3(*R*),4(*S*),8(*S*),9(*R*)]; cinchonidine X=H, [3(*R*),4(*S*),8(*R*),9(*S*)]; 80 quinidine [3(*R*),4(*S*),8(*R*),9(*S*)].

As in earlier work[111] the negative effect of 1-pentanol on stereoselectivity was observed. Influence of the alcohol may be due to competitive interaction with functional groups in the ions, thus disturbing the selective interaction producing the diastereomeric ion-pairs. The nature of the anion in the mobile phase was especially

influential on the retention and stereoselectivity of carboxylic acids.[113] Table 6.4 shows the results which were obtained with different counter ions, all having a distance

Table 6.4. Influence of counter ion structure on the stereoselective retention of 10-camphorsulphonic acid.

Chiral salt	Content (mol/l 10^4)	1-Pentanol (%, v/v)	α +/-
(+)-Alprenolol chloride	10.0	0	0.95
(-)-Quinine chloride	3.0	1	1.33
(-)-Quinine acetate	3.5	1	1.47
(+)-Qininidine acetate	3.5	1	0.77
(-)-Cinchonidine acetate	3.5	1	1.24

of two carbons between the hydroxy and amino functions. (+)-Alprenolol (78), which has both of these functional groups incorporated into a straight chain gives a fairly low separation factor. Better α values were obtained when quinine and other cinchona alkaloids were used. The latter

counter ion compounds contain a tertiary amino group in a ring system. It was suggested that improved stereoselectivity was due to the interaction of bulky and rigid groups in the vicinity of the chiral centre in the counter ion which could possibly increase the differential interactions with the enantiomers. In this work, enantiomeric camphor derivatives, mandelic, carboxylic and amino acid derivatives, were resolved, and structure/resolution correlations were made. Carboxylic acids, such as 2-phenylpropionic acid and naproxen with the aromatic ring structure, and a methyl group attached to the asymmetric centre, could not be separated by using cinchona alkaloid counter ions. A polar function such as a phenoxy group near to the chiral centre appears to be necessary for stereoselective retention. The fact that enantiomers of N-(1-phenylethyl)phtholmic acid were separable indicated that the carboxy group need not be directly bonded to the asymmetric carbon atom.[113]

Recently quinine and other cinchona alkaloids have been immobilised on silica and used as CSPs.[115] Acetylquinine has been used as the solid phase in combination with a chiral eluent.[116] A low stereoselectivity was observed for naproxen without a chiral additive in the dichloromethane mobile phase. The presence of an amine, dimethyloctylamine, in the mobile phase decreased the retention without affecting selectivity. Adding quinidine (ie., the same selector as in the stationary phase) drastically decreased stereoselectivity. However, using quinine as the counter

ion gave a significant improvement in stereoselectivity. The explanation for this is due the fact that quinine and quinidine are diastereomers (Fig 6.10), and generally yield chromatographic systems with reversed retention order for enantiomers. A possible explanation for the observed stereoselectivity with quinine in the mobile phase is that the chiral stationary phase binds to the (+)-naproxen stronger than (-)-naproxen, and that the quinine molecules interact more strongly with the (-)-naproxen and accelerate its elution through the column.

Ion-pair chromatography is a flexible technique with several possibilities to regulate retention and selectivity. The counter-ion character and concentration, as well as the addition of alcohol and protolytes to the mobile phase, can be used to control the resolution. A change of the stationary phase can also be used to effect both the retention and the stereoselectivity.

6.5 COMPUTER ASSISTED MOLECULAR MODELLING.

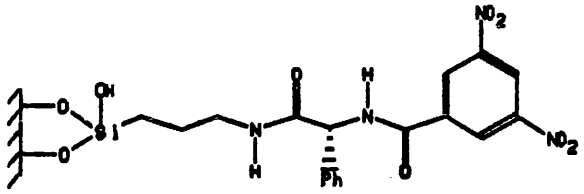
One of the most useful tools for the study of interaction mechanisms of CSPs is computer assisted molecular modelling (CAMM). With the aid of powerful software packages, it is possible to visualise and optimise the structure of possible diastereomeric complexes. Optimisation in this context means the computation of structural parameters required for favourable intermolecular interactionss between the bonded chiral

selector and the analyte enantiomers. This knowledge permits an approvement in the design of the CSPs, but at present in undertaking such computations some assumptions are made. For example, solvent effects are typically ignored, although it is known that the molecular size and shape of solvent molecules in the stationary zone occupied by the virtually stationary solvent, can effect enantioselectivity.[121] The case is further complicated when mixed solvents are used since it is not necessarily correct to assume that the stagnant zone has the same composition to the free moving eluent.

At present CAMM systems that will indicate the appropriate CSP for a given separation are far from perfect, but with ever increasing computing power and software sophistication, the ability of CAMM systems to predict stable conformations and interaction forces, and consequently the ability to explain observed enantiomeric discrimination will increase.

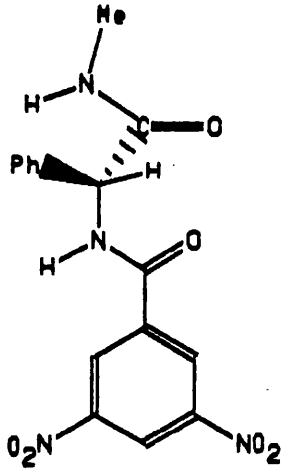
Lipkowitz and co-workers[122] made a detailed study of the covalent Pirkle CSP, *N*-dinitrobenzoyl-(*R*)-phenylglycine (CSP17). Using W. Clark Stills program and other supporting software, they established that the favoured conformation was *syn* rather than *anti* about the glycine's C-N bond (CSP 18), a difference of ca 21 kJ mol^{-1} existing between the two forms. Lipkowitz[123] also correctly predicted that the (*S*)-isomer of trifluoroanthrylethanol would form the more stable association complex with CSP 17, and thus be more strongly retained. However, their prediction differs

significantly from that proposed by Pirkle,[124] who suggested that different conformations were adopted by the two enantiomers when associating with the CSP.



CSP 17

Similar CAMM studies by Bridger et al[125] of CSP 19 and 20, derived from *N*-formyl-(*S*)-phenylalanine and nitrophenyl-(*R*,*R*)-tartramide respectively, have also been undertaken. Armstrong et al[126] studied the complexes of β-

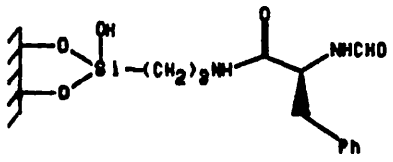


CSP 18

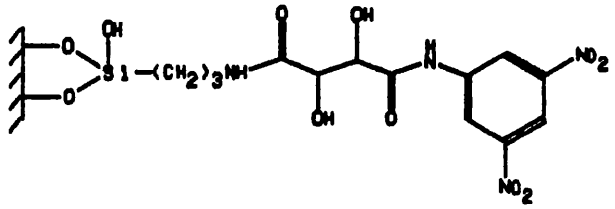
cyclodextrin with (*R*)- and (*S*)-propranolol, which required molecular structures of greater rigidity and complexity to

be modelled.

Despite the comparative ease of computing the separate conformations of each association complex, it is often time consuming, and more difficult to compute reliably the energy surface of the association complex together with



CSP 19



CSP 20

interactions as a result of weak intramolecular forces. The association complexes are compared visually in such a way that the physical modes are used. The interatomic distances can be compared and the angles at which hydrogen bonds may arise can also be considered. Yet despite such limitations, alternative mechanisms can often be discerned and placed in order of probability. With the imminent introduction of the transputer, such computations will, it is certain, become both faster and more reliable.

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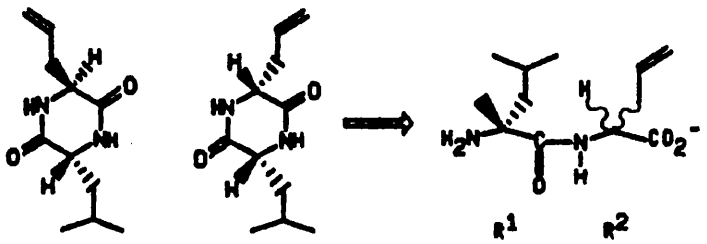
RESULTS AND DISCUSSION

CHAPTER SEVEN.

7.1 CONVERGENT SYNTHESIS OF THE TARGET CYCLIC PEPTIDES.

The initial target compounds, cyclo-leucyl-(allyl)glycine (**13**, **14**) (Scheme 7.1), were synthesised as a diastereomeric mixture in order that a comparative test compound could be used subsequently for evaluation in HPLC

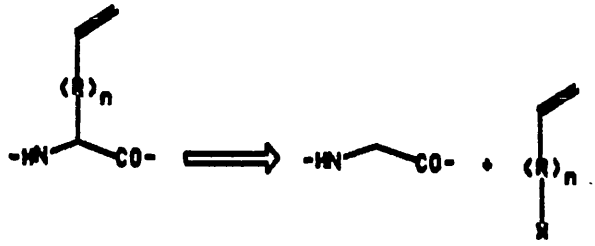


13 **14**

Scheme 7.1

separations. Both **13**, **14** are derived from a linear peptide of the type shown above, which is compounded from two amino acids, R^1 being an L-amino acid, L-leucine, and R^2 being an amino acid of either L- or D-configuration. L-Leucine was chosen, from the twenty constituent amino acids, as together with alanine it is generally free from those side reactions[1] which might interfere with the successful realisation of the target compounds. This lack of by-product formation obviates the need for tedious purification of the intermadiates, and also reduces the opportunity for racemistion to occur. The component R^2,

which possesses an unsaturated terminal side chain required for eventual polymer attachment, is a glycine residue modified by an alkylation reaction (Scheme 7.2). The alkylation of glycine synthons provides an attractive route

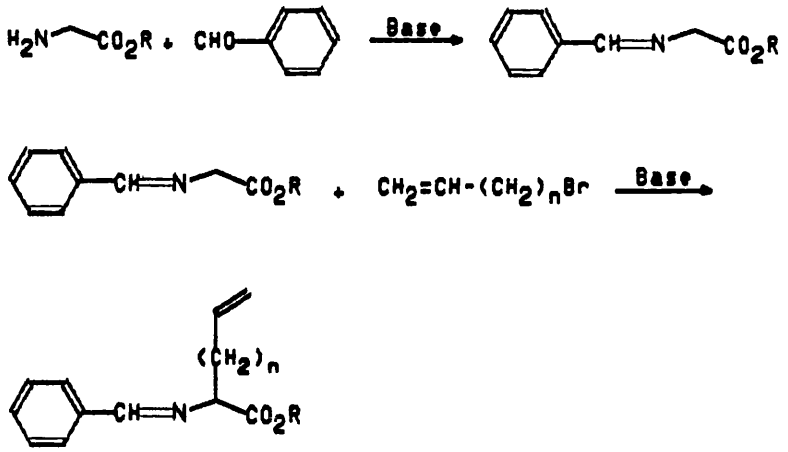


n=any number
X=halide

Scheme 7.2

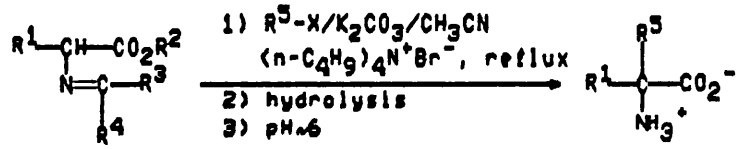
for the synthesis of structurally diversified amino acids, especially if both L- and D-isomers are required. The alkylation procedure chosen in this study is based on that reported by Stork *et al*,[2] and involves the synthesis of a Schiff base, derived from benzaldehyde and glycine esters, which can be alkylated in the presence of strong bases under anhydrous conditions (Scheme 7.3). Other literature routes are available, notably those of O'Donnell,[3-5] who synthesised the alkylated glycine residue via alkylation of either ketimine or aldimine derivatives (Scheme 7.4).[5] These alternative methods were not evaluated in view of the success of the first chosen procedure described above.

The dipeptide intermediate, t-butyloxycarbonyl-leucyl-(allyl)glycine methyl ester (11), was sythesised by each of the three methods shown (Scheme 7.5). In addition a fourth



n=any number
R=alkyl

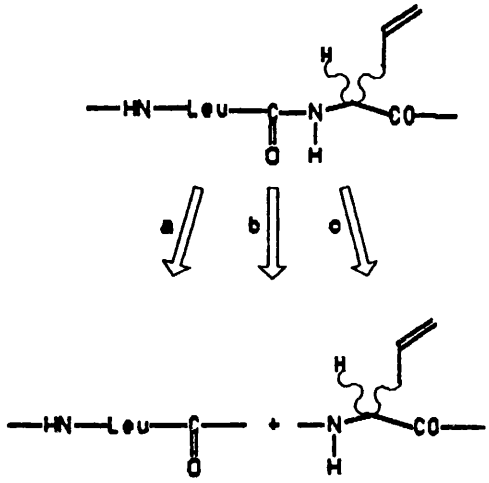
Scheme 7.3



R^1=H; R^2=C_2H_5; R^3=C_6H_5, H; R^4=C_6H_5, C_6H_4Cl; R^5=CH_2=$CH(CH_2)_n$-X (n=any number, X=halide).

Scheme 7.4

method was tried in which the peptide bond was formed with the aid of the coupling reagent dicyclohexylcarbodimide (DCCI). The usefulness of this reagent was first determined by synthesising the simple dipeptide, t-butyloxycarbonyl-leucyl-leucine mehyl ester (3). However, it was found that this peptide was invariably contaminated by dicyclohexyl urea, formed in the course of the reaction. This problem may be overcome by using water soluble carbodimides such as N'-(4-diethylamino cyclohexyl)- (Fig 7.1a) or N-ethyl-N'-

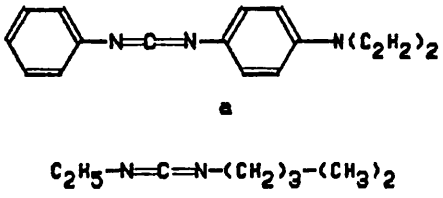


a=Mixed anhydride method

b=Active ester method

c=Azide method

Scheme 7.5



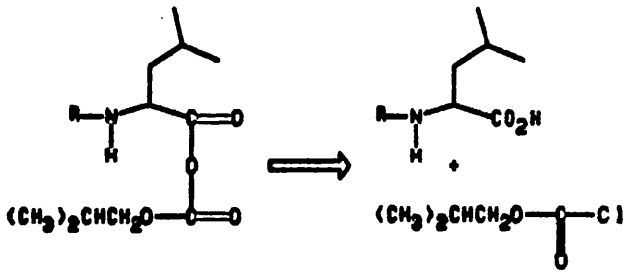
a

C_2H_5–N=C=N–$(CH_2)_3$–$(CH_3)_2$

b

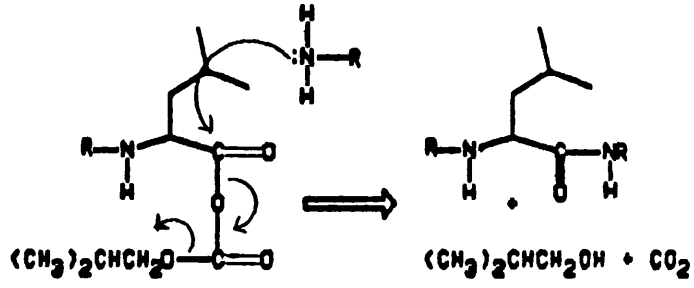
Fig 7.1

(3-dimethylamino propyl)-carbodimide (Fig 7.1b),[5,6] but in view of the success of the other methods detailed below, this fourth pathway to (*11*) was not pursued further. Of the methods finally used, method a involved the formation, *in situ*, of a reactive mixed anhydride intermediate, using isobutyl chloroformate (IBC),[8] of the amino-protected leucine residue (Scheme 7.6). The main advantage of this method is that the main by-products are the corresponding alcohol, isobutanol, and carbon dioxide, so facilitating



Scheme 7.6

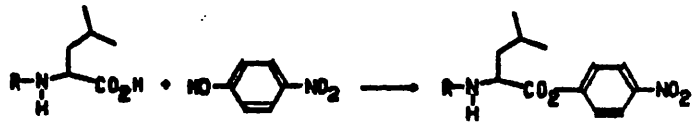
work up of the required product (Scheme 7.7). IBC was chosen on the basis of claims made by Vaughn et al[8] that this alkyl chloroformate was superior to a number of



Scheme 7.7

other alkyl chloroformates in giving the best yield, a claim later confirmed in studies by Anderson et al.[9]

Method b involved the synthesis of an activated ester intermediate, a para-nitrophenyl ester[10] of the amino-protected leucine residue (Scheme 7.8). The active ester was subsequently coupled with the alkylated glycine residue to afford the required dipeptide. Various other active ester intermediates, such as the cyanomethyl ester[11] or penta-halide aryl esters (eg., pentachloro/fluoro phenyl



Scheme 7.8

esters),[12,13] could have been used in this synthesis, but para-nitrophenyl esters were chosen due to their proven usefulness in peptide synthesis, most notably in the synthesis of the nonapeptide oxytocin (Fig 7.2),[14] formed by the stepwise elongation of the peptide chain by the addition of single residues.[15]

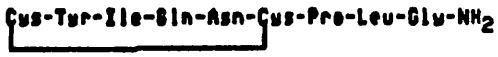
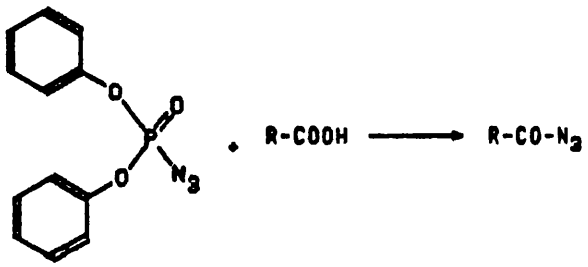


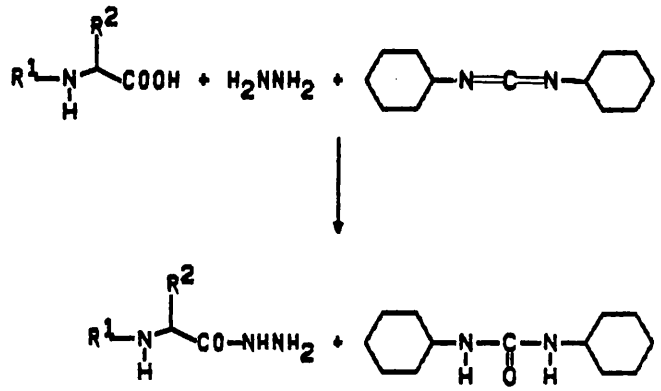
Fig 7.2

Method c involved the formation of a reactive intermediate, *in situ*, using diphenylphosphoryl azide (DPPA) (Scheme 7.9).[16] DPPA converts carboxylic acids into



Scheme 7.9

the corresponding acid azide and subsequent nucleophilic attack by the amino component affords the required dipeptide. It is possible to form the acid azide via the hydrazide intermediate, but this would require isolation and purification of the intermediate. In addition, this method[17] also requires the use of DCCI as a coupling reagent/dehydrating agent, and persistent contamination would again be anticipated (Scheme 7.10). Using DPPA simplifies the synthesis as the reactive intermediate is formed *in situ* and requires no isolation. Cylcisation of the dipetide was acheived using the method of Nitechi *et al.*[18]



Scheme 7.10

An alternative strategy for the preparation of the initial target compounds (*13*, *14*) involves the synthesis of the linear dipeptide (*11*) such that the alkylated glycine residue is the first residue counting from the N-terminus (Fig 7.3). This route involves the synthesis of alkylated glycine residues possessing a free carboxylic acid. The

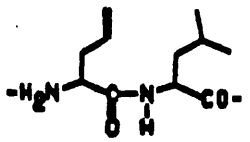
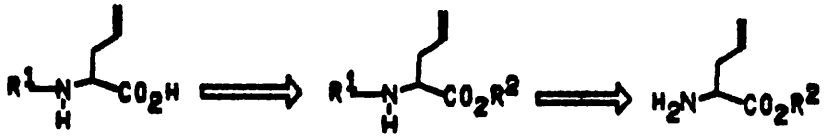


Fig 7.3

retro-synthetic scheme is in Scheme 7.11. It would have been equally suitble had not the saponification of the ester to the free acid given only moderate yields.



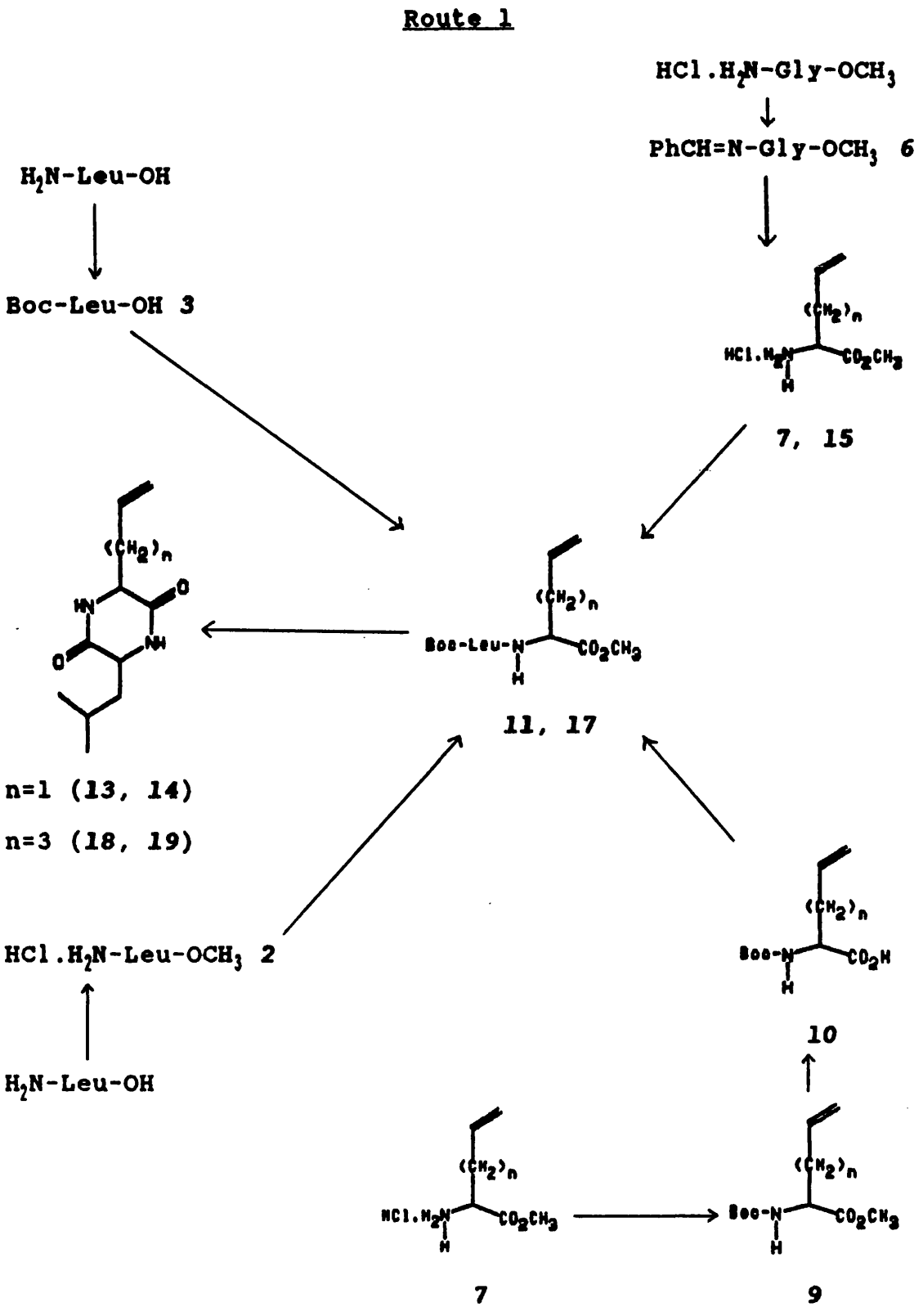
R^1=amino protecting group

R^2=alkyl

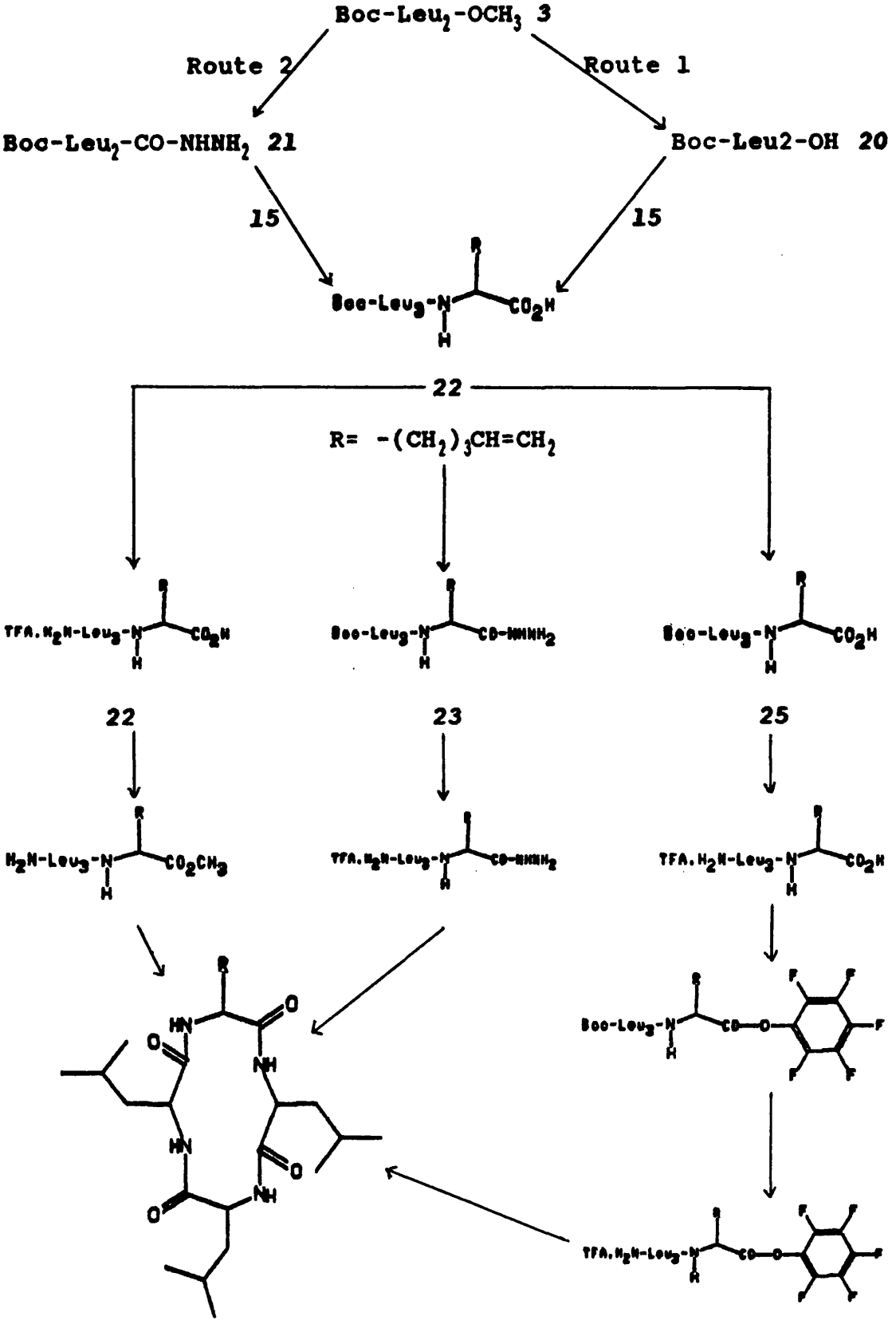
Scheme 7.11

The synthesis of the next target compound (Scheme 7.13), a cyclic tetrapeptide (24), could be achieved by several methods. These involve the formation of a linear tetrapeptide (22) prior to cyclisation to give the required product. Two routes towards the linear tetrapeptide were attempted. Route 1 involved the hydolysis of the di-leucine methyl ester peptide (3), using the method of Iselin et al,[19] to give the corresponding carboxylic acid. Had this method worked satisfactorily, the mixed anhydride method[8] would have been used to form the tetrapeptide. However, a very poor yield resulted from the hydrolysis step and so, this second stage was not attempted.

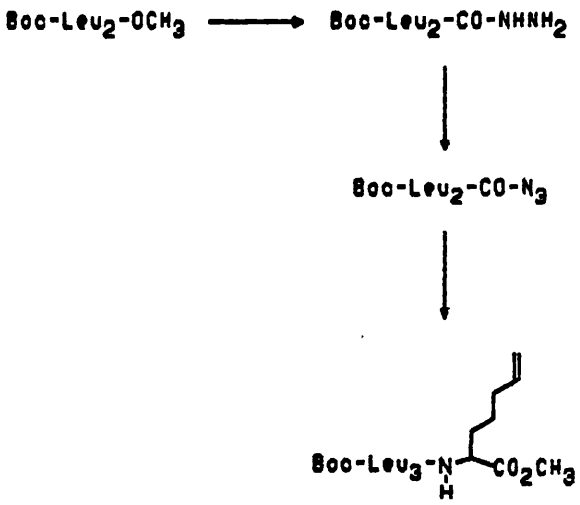
Scheme 7.12. Convergent synthesis of cyclopeptides.



Scheme 7.13. Convergent synthesis.



Route 2 involved the synhesis of a hydrazide intermediate which could then be converted to the azide using sodium nitrite[20] or butyl nitrite[21]. Subsequent coupling with the alkylated glycine residue would afford the linear tetrapeptide (Scheme 7.14). For the cyclisation of the linear tetrapeptide several options are possible, and are discussed in more detail in CHAPTER EIGHT, section 8.11 of this thesis.



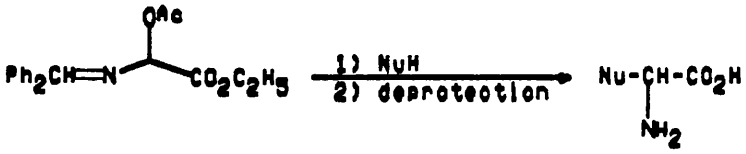
Scheme 7.14

7.2 ALTERNATIVES TO THE SYNTHESIS OF UNSATURATED GLYCINE DERIVATIVES.

Subsequent to the work described in this thesis, there has appeared in the open literature, several alternative syntheses of allyl glycine (7).[22-21]

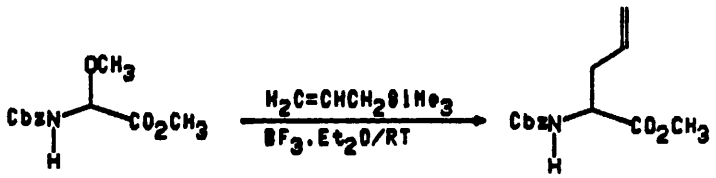
In their study of the synthesis of functional amino

acids by reaction of an electrophilic glycine cation equivalent with neutral carbon nucleophiles, O'Donnell and Bennett[22] synthesised diphenylmethyl-D,L-(allyl)glycine ethyl ester. Thus reaction of an α-acetoxyglycine Schiff base with allyl trimethylsilane, in the presence of $TiCl_4$, gave the α-substituted amino acid in yields of up to 44%. The acetate derivative has been described as "a versatile cationic equivalent, being easily prepared and containing several structural subunits of interest for bond formation to the α-carbon." Reactions are of the general type shown in Scheme 7.15.

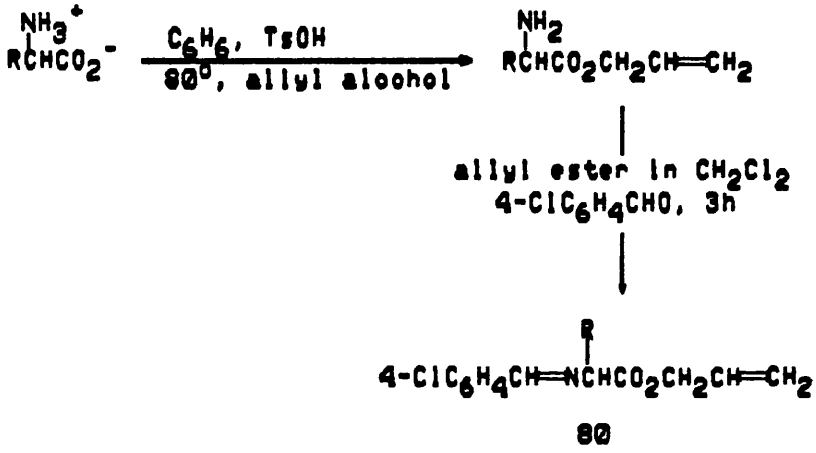


Scheme 7.15

This work was confirmed by Castelhano et al[23] who studied the synthesis of α-amino acids with β,γ-unsaturated side chains. Under Lewis acid conditions *N*-carbobenzyloxy-(allyl)glycine methyl ester was synthesised with *ca* 80% conversion of the starting material to the product (Scheme 7.16). Van der Werf and Kellogg[24] studied the synthesis of α-allyl-α-amino acids by means of a palladium catalysed intramolecular re-arrangement via a variation of the Carroll reaction.[25] Various L-α-amino acids were converted to the allylic ester, and then to the imines (80) (Scheme 7.17). The overall yield for these steps was 75-80%.



Scheme 7.16



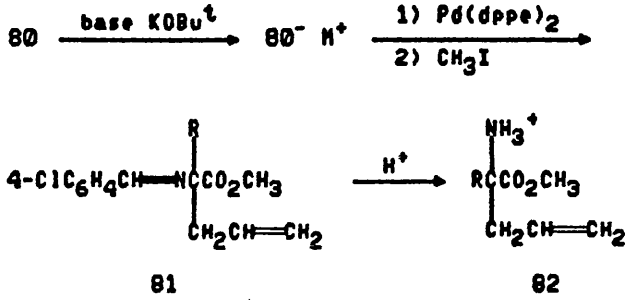
R=H

Scheme 7.17

Compound 80 re-arranged cleanly to 81 (Scheme 7.18) in the presence of 1,2-bis-diphenylphosphinoethane palladium(0) ($Pd(dppe)_2$). Quenching the carboxylate salt with CH_3I and subsequent *in situ* hydrolysis of the imine, afforded the methyl esters (82) as stable compounds. No reaction occurs in the absence of catalyst. Yields for the re-arranged product from the imine range from 65-90% depending on the base used.

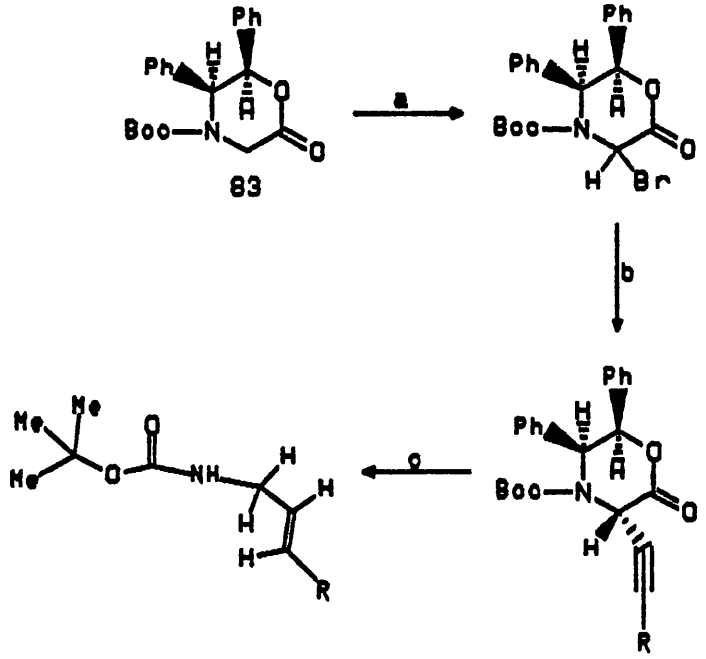
All the alternatives described above afford the racemic product. A recent paper, by Williams and Zhai,[26] describes a versatile, stereocontrolled, asymmetric

synthesis of *E*-vinyl glycine derivatives (Scheme 7.19). Yields ranged from 70-80% with enantiomeric excess (ee) of



R=H

Scheme 7.18



a:- NBS/CCl_4; b:- R_3Sn-C≡C-R, $ZnCl_2/CCl_4$, reflux; c:- Na/NH_3/EtOH

R=CH_3, n-C_3H_7, n-C_6H_{13}, $(CH_2)OSiMe_2Bu^t$.

83 tert-Butyl-6-oxo-2,3-diphenyl-4-morpholine (Aldrich Chem. Co.).

Scheme 7.19

55-70% for the Na in liquid ammonia/EtOH reduction. Reduced yields were obtained using Li as reductant, but ee increased to 98%. This method is of potential use in a modified form for future synthesis of unsaturated glycine derivatives with a terminal double bond. The proposed route would be similar, except that a trialkyl tin derivative would be the carbanion source as shown in Fig 7.4. Such tin

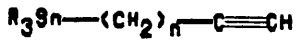
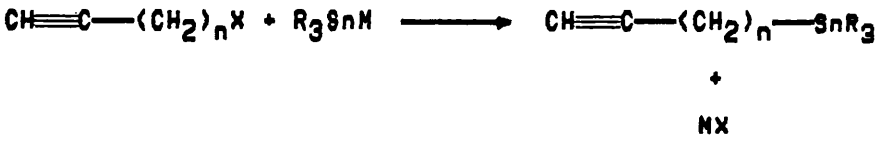


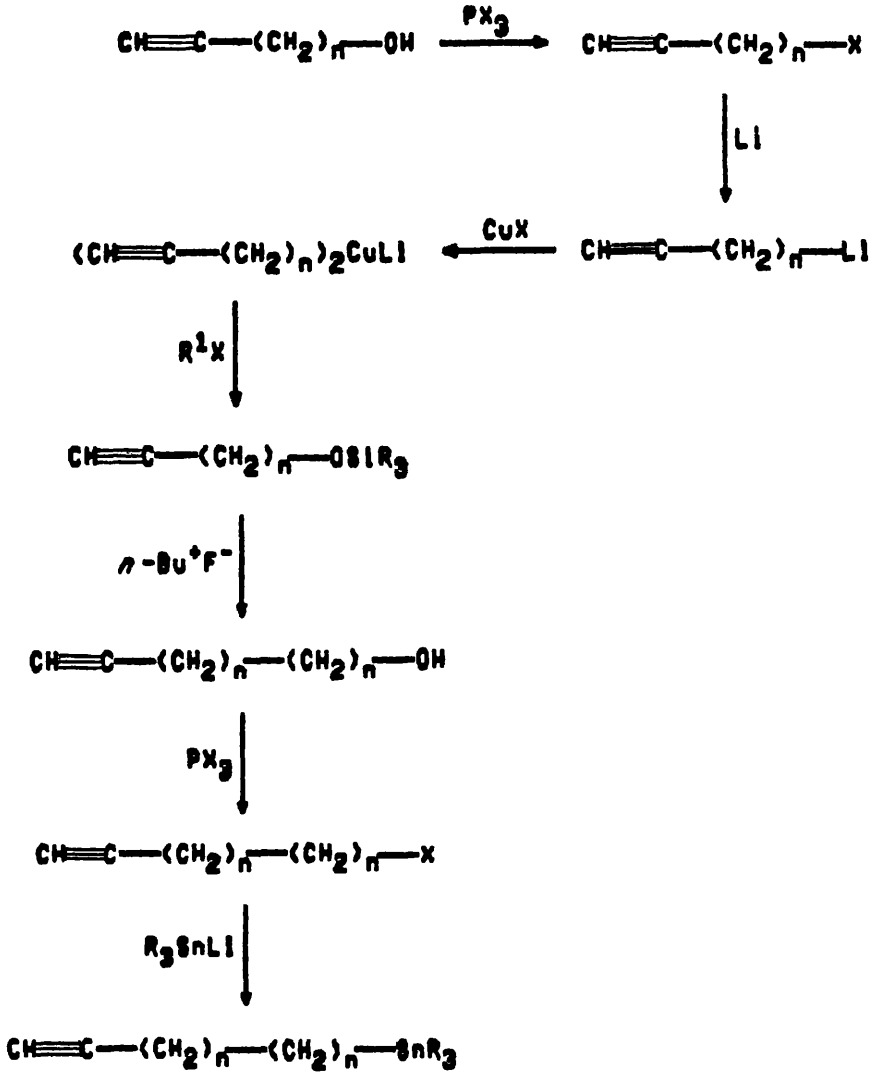
Fig 7.4

compounds can be easily synthesised beginning from the corresponding unsaturated alcohols, as outlined below using the methodolgy described by Seyferth[27] (Scheme 7.20), and Corey and House (Scheme 7.21).[28,29] Protection of the hydroxyl function can be achieved by conversion to the trimethylsilylether.[30]



X=halide

Scheme 7.20



X=Cl, Br

R^1=X—$(CH_2)_n$—$OSiR_3$

Scheme 7.21

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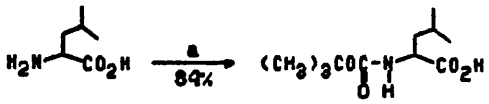
CHAPTER EIGHT.

RESULTS AND DISCUSSION.

8.1 Synthesis of tert-butyloxycarbonyl-L-leucine (*1*) and L-leucine methyl ester hydrochloride (*2*).

The synthesis of tert-butyloxycarbonyl-L-leucine (*1*) (Scheme 8.1) was achieved in 84% yield using the method of Schwyzer et *al*.[1] Elemental analysis of the product indicated

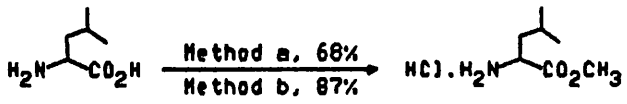


Scheme 8.1. a) Dioxane/H_2O, Na_2CO_3, $(Boc)_2O$, 0^0C-->RT

that a mono-hydrate had been isolated, and the product was stored in this form. Prior to further use in synthesis, samples of the product were first dried by azeotropic distillation of water with benzene, which had been dried with sodium-lead alloy, and then held *in vacuo* for ca 24h before use.

L-leucine methyl ester hydrochloride (*2*) was synthesised (Scheme 8.2) by either one of two methods. Method a involved the use of 2,2-dimethoxypropane (2,2 DMP),[2] which serves both as a de-hydrating agent, and as a source of methoxy-groups (Fig 8.1). This has the advantage that it does not require the use HCl gas or specially prepared solvents. Both methanol and acetone can be easily

removed, and the method is well suited to small scale



Scheme 8.2. Method a:- 2,2 DMP, conc HCl, RT, 48h; method b:- MeOH, dry HCl gas, RT.

preparation, where the cost of the 2,2 DMP is not prohibitive. Method b (Scheme 8.2) was based on the procedure given by Schott et al,[3] in which dried HCl gas was bubbled through a suspension of L-leucine in dried methanol. The procedure gave good yields of very pure product, and had the advantage over method a that it could be scaled up without difficulty and prohibitive costs.

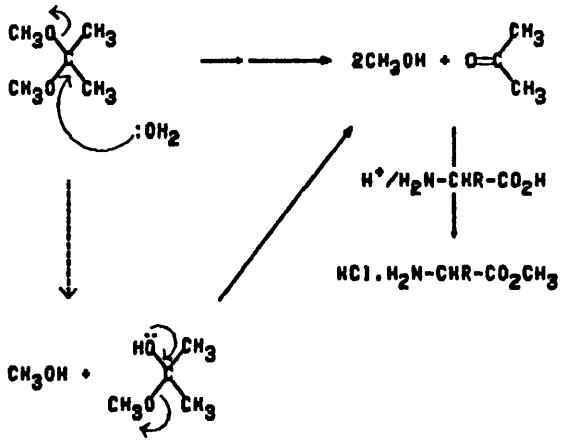
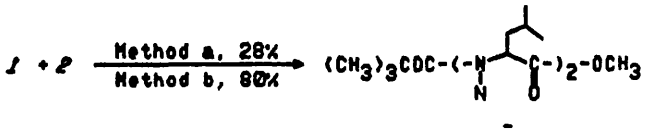


Fig 8.1

8.2 Synthesis of oligo-L-leucine peptides.

The synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucine methyl ester (3) was achieved, for comparative purposes, by two methods a and b. The two methods used to synthesise (3) are shown in Scheme 8.3. In method a, peptide bond formation was achieved with the aid of dicylclohexylcarbodimide (DCCI),[2,5] and in method b via the



Scheme 8.3. Method a:- DCM, disopropylamine, DCCI, HOBt, 0°C-->RT; method b:- THF/DMF, base, IBC, -15°C, 6-24h.

formation of a reactive mixed anhydride intermediate, formed from isobutylchloroformate (IBC).[6] Of the two methods, method b gave better yields. There was also a large difference in the measured optical rotation of the compounds produced by the two methods ($[\alpha]_D$= -26 and -49.8 for (3) from method a and b respectively). Papers published by Nitechi et al,[7] and Suzuki et al,[8] give optical rotations of -50.4 and -55 respectively for (3), both groups using the carbodimide method of synthesis, but Shields et al[6] reported a measurement of only -25.7 using the mixed anhydride method. It would appear ^Xxat the pure compound has a $[\alpha]_D$ value of ca -50, and can be prepared by either procedure. However, lower values presumably reflect impure material either due to racemisation or dicyclohexylurea

(DCU), which is inherently difficult to remove satisfactorily. The wide melting-range of (3) produced by method a (100-105°C), is also considerably lower than that quoted in the literature (136-137°C; 141-142°C; 132-133°C),[6-8] and is also indicative of persistent contamination.

8.2.1 Mechanism of peptide bond formation with dicyclohexylcarbodimide.

The mechanism of this coupling reaction was elucidated by Smith et al[9] (Fig 8.2). Activation of the carboxylic acid group is initiated by the transfer of its proton to the double bond of the carbodimide (I). The resulting compound (II), may react in two different ways. O->N Acyl migration can occur with the formation of an N-acyl urea (III) if no further protons are available. In the presence of excess acid, the second addition of a proton takes place, and O->N acyl migration is prevented so that (IV), a symmetrical anhydride, is formed together with a di-substituted urea, DCU. It is the anhydride which appears to be the species that reacts with the amine to give the desired product (V), and an acid, which can be "re-cycled" in to the reaction. The product (V) might also be formed from (II), but there exists here the opportunity for racemisation via oxazolones/azalactones.[10] The mechanism can be shown as illustrated in Fig 8.3, where X is an electronegative group of the type present in activated intermediates for peptide synthesis. Enhancement of the electrophilic character of the carbonyl carbon C5 facilitates the attack of the

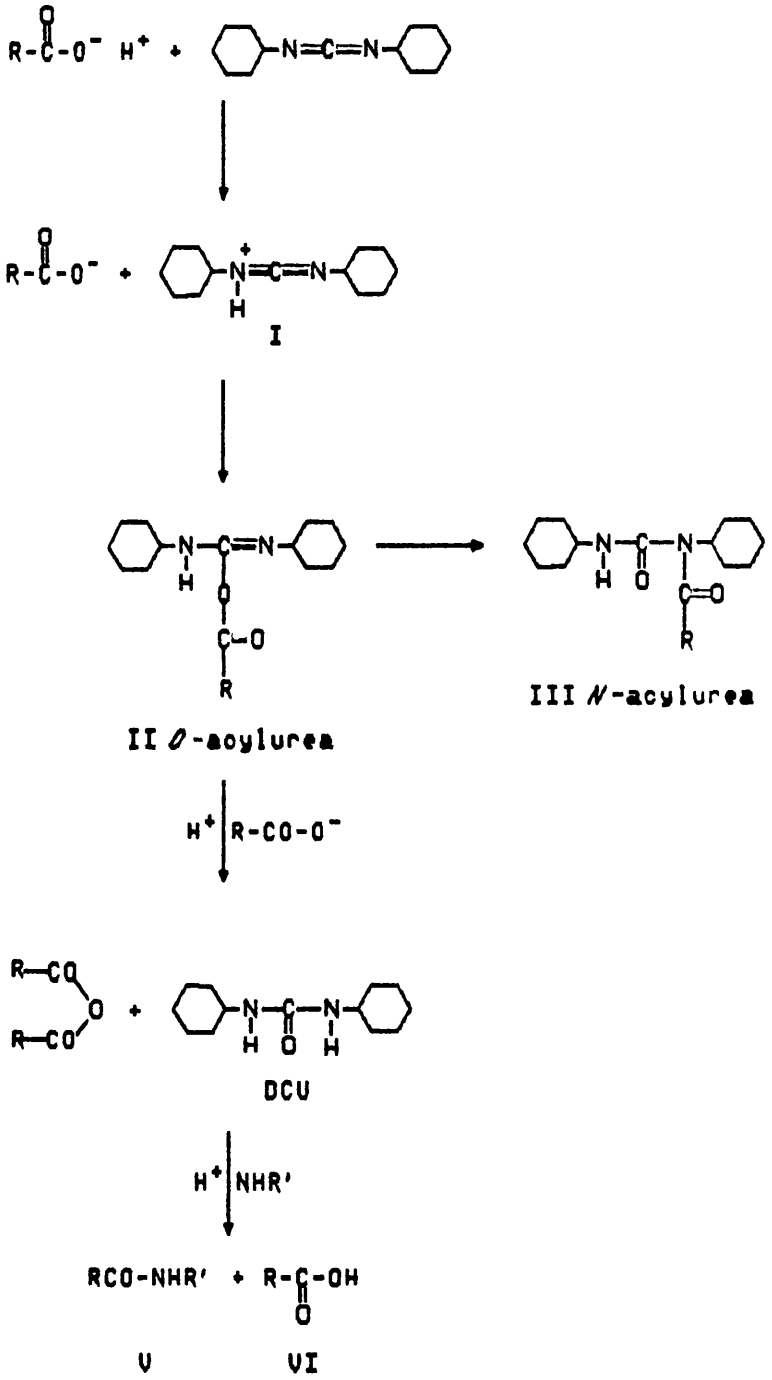


Fig 8.2

carbonyl oxygen Cl. The oxazolone formed has a strong tendency, especially in the presence of base, to lose a proton at carbon C4, thus giving the resonance-stabilised

anion of oxazolone, leading to racemisation of the amino acid (Fig 8.4).

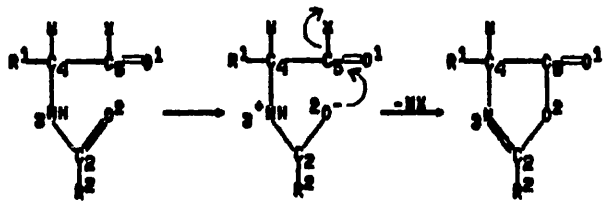


Fig 8.3

Oxazolones are good acylating agents, and could be used for the activation of the carbonyl component, yet delocalisation of the negative charge in the de-protonated

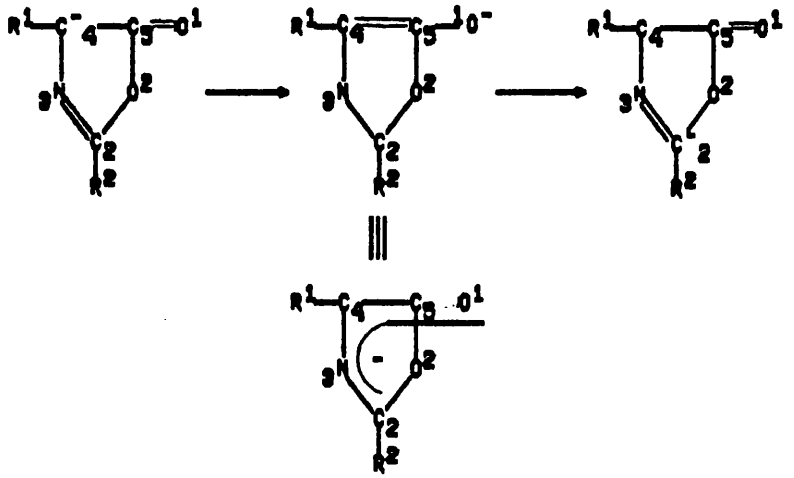


Fig 8.4

intermediate provides them with sufficient lifetime to endanger the chiral purity of the product. The formation of oxazolones has been demonstrated by Williams et al,[11] using infrared spectroscopy to monitor the characteristic carbonyl frequency (1832cm^{-1}) observed when benzoyl-L-

leucine-para-nitrophenol ester was exposed to tertiary amines (Fig 8.5). Racemisation through oxazolone intermediates is influenced by a number of factors, such as the nature of the amino acid involved, the solvent used in the reaction, or the presence of tertiary amines. The acyl group on the amine nitrogen appears to play a decisive role

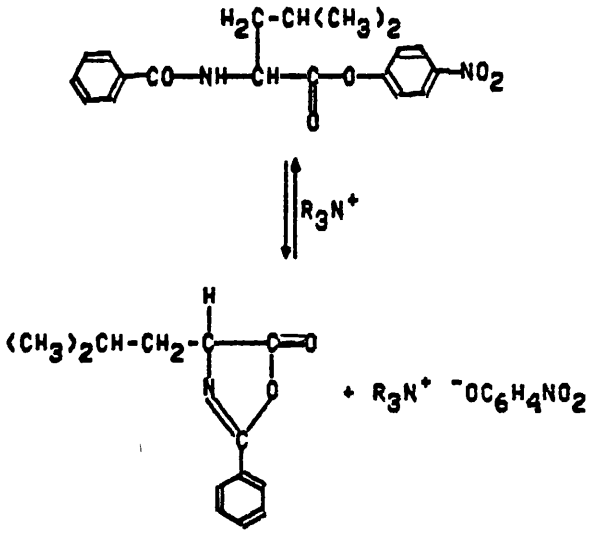
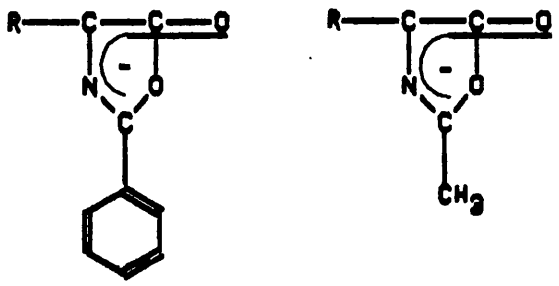


Fig 8.5

in the conservation or loss of chiral purity. For example, under identical conditions, benzoylamino acids are more extensively racemised than acetylamino acids.[12] Such differences seem to be related to the electronic forces operating on the acyl-group. Beyond the formation of oxazolones, the *N*-acyl substituent of the oxazolinone can also affect the acidity of the hydrogen atom on the chiral centre ie., the stability of the anion produced in the proton abstraction by bases is enhanced by the electron with-drawing effects of the acyl-group (Fig 8.6). Oxazolone



more stable anion less stable anion

Fig 8.6

formation is probably not as important as the electronic effects of the substituent of the oxazolinone, including those in the *N*-acyl-group, in determining the extent of racemisation. Oxazolones can however, be obtained in optically active form,[13] and if immediately trapped by good nucleophiles,[14] can yield optically active products.

The influences of the *N*-acyl-group on the stability of the anion generated through proton abstraction from the oxazolone can range from extreme stabilisation, found in the formyl and trifluoroacetyl-groups, to pronounced destabilisation, shown by the benzyloxycarbonyl, tert-butyloxycarbonyl (Boc), and other alkyloxycarbonyl groups. It had been generally assumed that benzyloxycarbonylamino acids, and in general, amino acids protected by a urethane type blocking group, did not produce oxazolones, and were therefore resistant to racemisation during activation and coupling. However, isolation[15] of optically pure oxazol-(4H)-ones eg., from the reaction of tert-butyloxycarbonyl-L-valine with water soluble carbodimides, contradicts such assumptions, and suggests that the beneficial effects of

urethane type protecting groups rests on the electron release provided by them, and on the resulting destabilisation of the anion which could be formed by proton abstraction (Fig 8.7). It is highly probable that in addition to the already mentioned mechanism of base-catalysed racemisation, other pathways also exist.

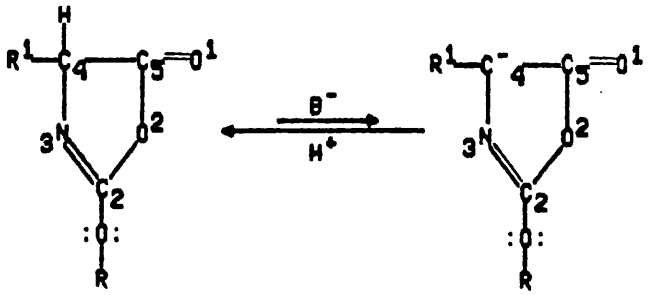


Fig 8.7

The role of bases, in at least some racemisation processes, is beyond doubt. For instance, it has been noted that there are advantages of free amines over amine salts in suppressing racemisation. So far less attention has been paid to the possibility of <u>intramolecular</u> base catalysis. Since O-alkylisoureas have pronounced basic character, it could be assumed that proton abstraction by a basic nitrogen atom in the O-acylisourea intermediates of the carbodimide mediated coupling reaction is possible (Fig 8.8). Alternatively enols stabilised by hydrogen bonding formation might play a role in such processes (Fig 8.9). This becomes analogous to the effect of excess acetic anhydride on optically active amino acids, where racemisation proceeds through enolisation of mixed

anhydrides (Fig 8.10).

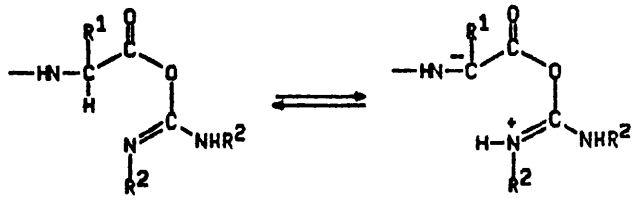


Fig 8.8

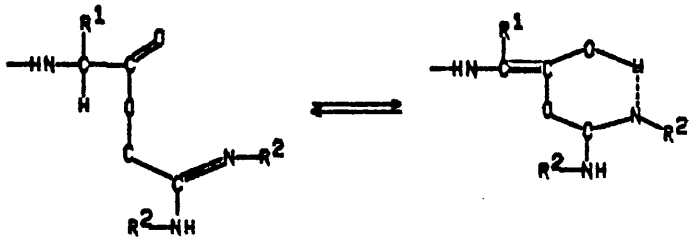


Fig 8.9

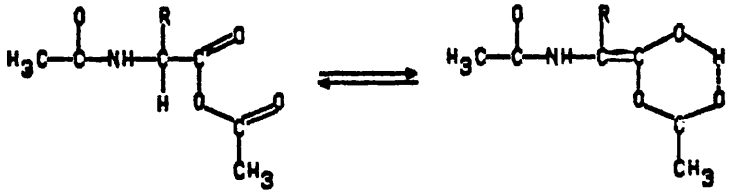
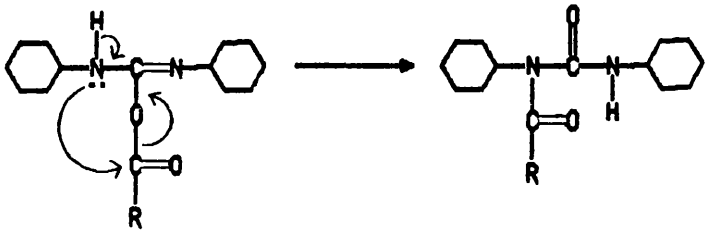


Fig 8.10

The re-arrangement of O-alkylisoureas (II) (Fig 8.11) to the *N*-acylurea (III) also "stops" the reaction, as the latter are not acylating agents. However, their formation can be minimised by performing the reaction at $0^{0}C$ or lower, and also by using non-polar solvents, such as DCM or MeCN.

A technical difficulty in the preparation of peptides using DCCI, referred to earlier, is the complete removal of the DCU formed when reactions are carried out in most

organic solvents except alcohols. This problem has been circumvented by the introduction of carbodimides which give water soluble urea products.[17]

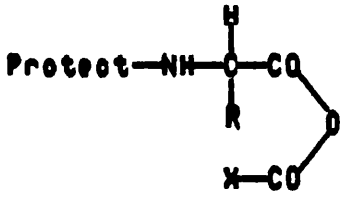


II III

Fig 8.11

8.2.2 Mechanism of peptide bond formation via mixed anhydrides.

The use of alkyl chloroformates as peptides bond forming reagents was independently proposed by three groups.[18-20] The method became popular due to its advantages of speed, yield, and relative purity of products. Previous methods of mixed anhydrides employed alkyl-groups of the type illustrated in Fig 8.12. In general, if X=CH_3 or phenyl, low yields were obtained, since, in the case of X=phenyl, the electron with-drawing effect rendered the carbonyl carbon of the benzoyl residue sufficiently electrophilic to compete in the acylation of the amine to a significant extent with the protected amino acyl moiety (Fig 8.13). For more satisfactory results, an electron releasing structure was required. This was partially fulfilled by the use of longer aliphatic chains, especially



Protect=protecting group; X=alkyl [CH_3, $(CH_3)_3C$, $(CH_3)_2CHCH_2$] or phenyl.

Fig 8.12

acids with branched chains eg., X=$(CH_3)_3C$, which gave good yields with little by-product formation. However, it may be reasonable to assume that steric hinderance also contributes to the formation of the required product.

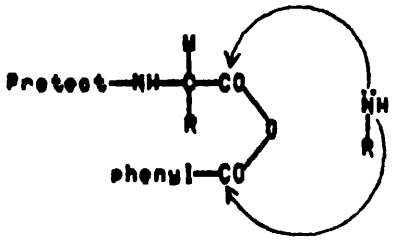
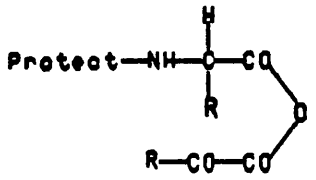


Fig 8.13

A different approach was proposed[18-20] in which the X-group was an alkoxy-group, the alkoxy-group being the electron releasing part of the molecule ie., replacement of carboxylic acids with half esters of carbonic acids (Fig 8.14). In the presence of an acid binding agent, such triethylamine (TEA), a rapid reaction occurs between the protected amino acid, or peptide, and the chloroformate, to afford a mixed anhydride, in which the reactivity of the



R=alkyl

Fig 8.14

carbonic acid carbonyl is diminished by the effect of the unshared pair of electrons on the neighbouring atom (Fig 8.15). Hence only little second acylation product, a urethane, can be expected (Fig 8.16). It is a particular

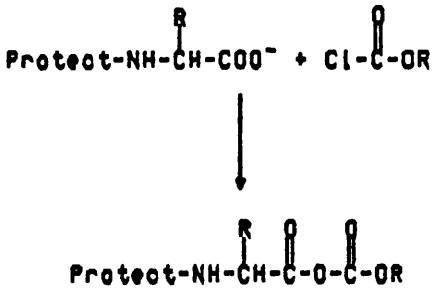


Fig 8.15

advantage of the method that the by-products formed in the decomposition of the leaving group are an alcohol and carbon dioxide, which do not interfere with the isolation of the protected peptide. Participation of the "wrong " carbonyl group in the acylation reaction was further reduced by the modifications proposed by Vaughn,[18] who introduced isobutylchloroformate (IBC) (Fig 8.17), one of the most widely used activating reagents in peptide

synthesis. In this method, the undesired attack on the carbonyl-group of the carbonic acid usually yields less than 1% of the urethane.

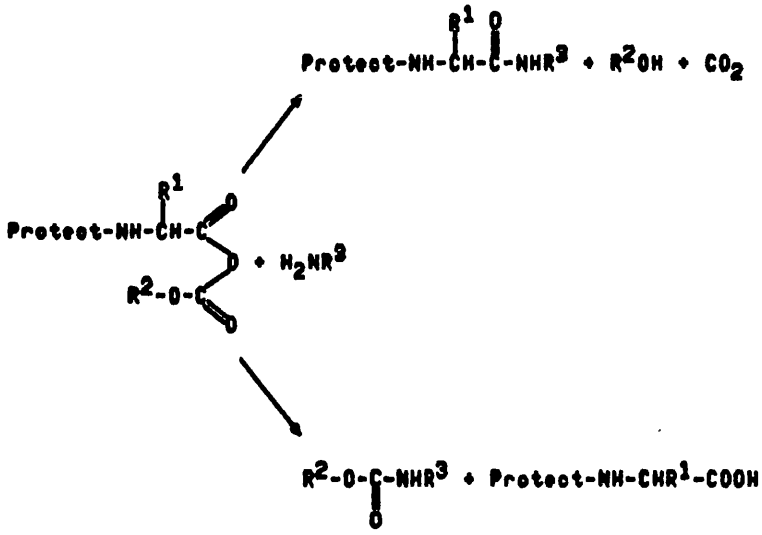
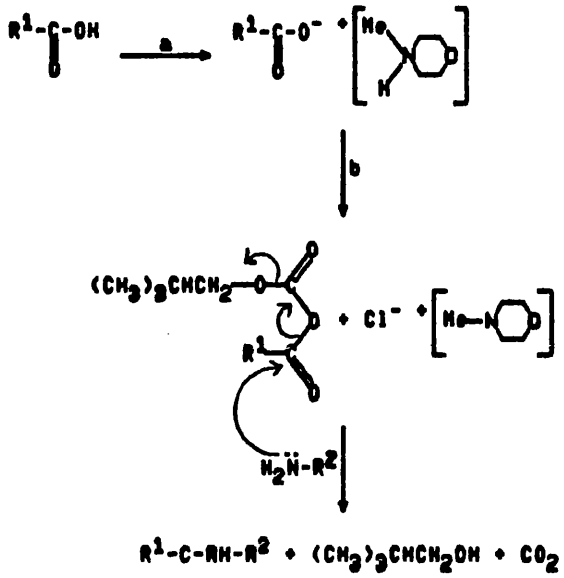


Fig 8.16

Fig 8.17

Formation of the mixed anhydride is practically instantaneous, even at low temperature (-15°C), as indicated by the immediate precipitation, if insoluble in the solvent used, of the corresponding hydrochloride salt of the base. The mechanism of the reaction is illustrated in Fig 8.18.

Factors which affect yield, and racemisation using this method have been reviewed by Anderson et al.[21] He states that the tertiary base used for mixed anhydride



a=base eg., *N*-methylmorpholine; b=IBC

Fig 8.18

formation of an acyl-amino acid or acyl-peptide with an alkylchloroformate is not merely a hydrogen chloride acceptor. It first reacts with the chloroformate to form a quaternary compound, which in turn reacts with the carboxylic acid. Methyl-amines give the fastest reaction, showing the importance of steric factors. However, methyl-amines also cause most racemisation, indicating that racemisation is also sterically influenced (Fig 8.19). The discovery that bases, such as *N*-methylmorpholine (NMM), gave rapid anhydride formation without racemisation in test cases, indicated racemisation to be a separate process from anhydride formation. Anderson et al[21] also state that triethylamine, although a good racemising agent, when used in excess, could be used without causing racemisation, if not in excess, and the precipitation of the HCl salt could

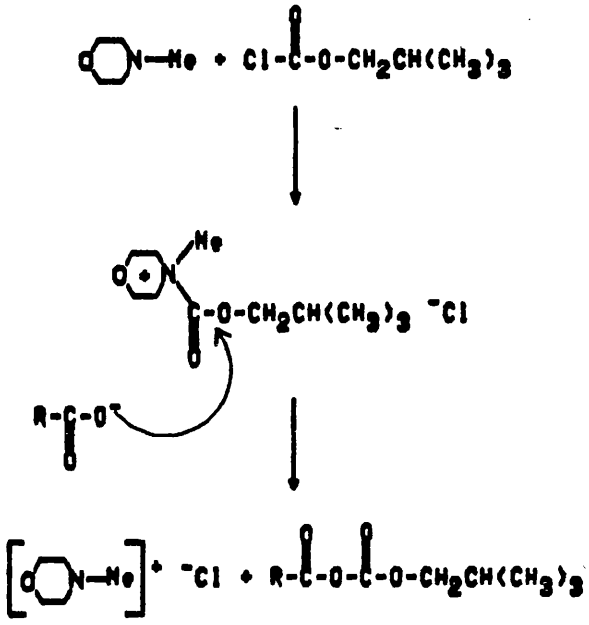


Fig 8.19

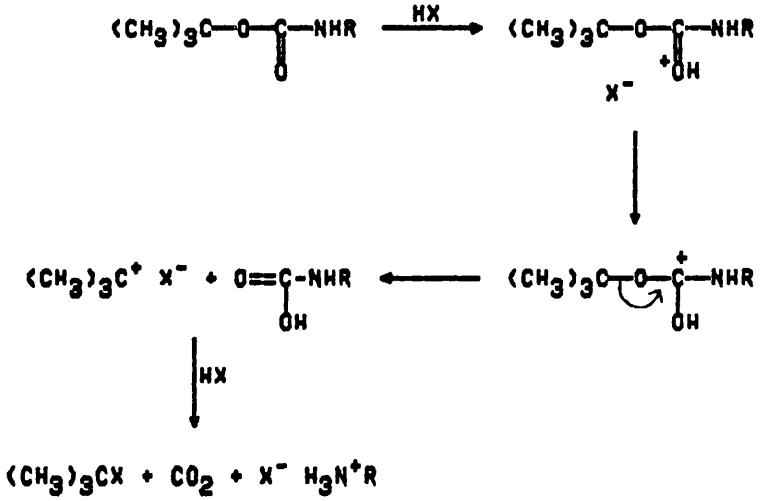
be used as an indicator for mixed anhydride formation.

In earlier reports Young and co-workers, and Vaughn,[22-25] noted that the mixed anhydride method had been reported to produce a substantial degree of racemisation. Anderson et al[21] showed that oxazolone formation was evident in the mixed anhydride method. However, other workers[21-27] suggest racemisation can be minimised if the reaction is performed at low temperatures (-15°C) and that no more time than necessary is allowed for the formation of the mixed anhydride. Applewhite and Nelson[28] suggest that "inverse" addition also reduces racemisation. According to this procedure, a solution of the salt of the protected peptide with a tertiary amine is added to a solution of the alkyl-chloroformate, which is used in excess (2-5 molar). In this way the basic carboxylate anion is present in the reaction mixture for a very limited period. However, Anderson et al[21]

have reported that the method of "inverse" addition appears to produce a substantial degree of racemisation, in the range of 3-6%, in the synthesis of benzoyloxycarbonyl-glycyl-L-phenylalanyl-glycine methyl ester.

8.2.3 Removal of the protecting group, tert-butyloxycarbonyl.

Cleavage of the Boc group was accomplished using trifluoroacetic acid (TFA)[29] (Scheme 8.4). The deprotected oligo-peptides were either precipitated from ether/pet ether, or the solvent removed *in vacuo*, and used without further purification or characterisation.

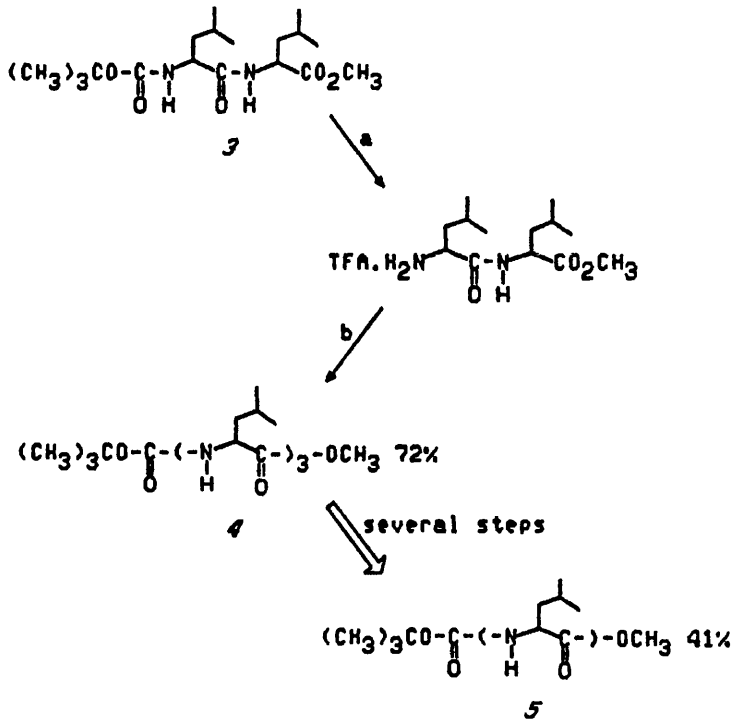


Scheme 8.4

8.2.4 Synthesis of tri- and tetra-L-leucine methyl ester peptides.

Synthesis of these oligo-peptides (4) and (5), was

achieved in a similar fashion to that of the oligo-di-L-leucine methyl ester (3) (Scheme 8.5). Yields, as expected



a:- TFA, 1h, RT; b:- base, THF/DMF, IBC, -15^{0}C, 24h.

Scheme 8.5

decreased with increasing peptide chain length due to steric crowding around the carbonyl group which the amino component attacks. Again a discrepancy is found in our measured optical rotations, and those reported by Shields et al[6] (Table 8.1). However, the identity of the product is confirmed by both NMR and MS data, with no sign of impurity.

Table 8.1. Comparison optical rotation of oligo-L-leucine peptides.

Boc-L-$(Leu)_n$-OMe

n	Measured value	Literature value
1[a]	-22.1	-24.0[1]
2[b]	-26.0	-50.4[7]; -55.0[8]; -46.9[d]
2[c]	-49.8	-25.7[6]
3[c]	-64.3	-21.7[6]; -75.1[d]
4[c]	-86.9	-16.4[6]; -92.6[d]

a:-Boc-L-Leu-OH; b:-Dicyclohexylcarbodimide method or variation of; c:-Mixed anhydride method; d:- G.M. Benora, A. Maglione and C. Toniolo; *Polymer*, 1974,15,767.

8.3 Synthesis of benzylidine glycine methyl ester (6).

The synthesis of benzylidine glycine methyl ester (6), was achieved by the method of Stork *et al*[30] (Scheme 8.6). The product, although synthesised in high yield, was slightly impure (NMR data), but this was considered suitable for direct use in the next synthetic step.

8.3.1 Alkylation of benzylidine glycine methyl ester with allylbromide.

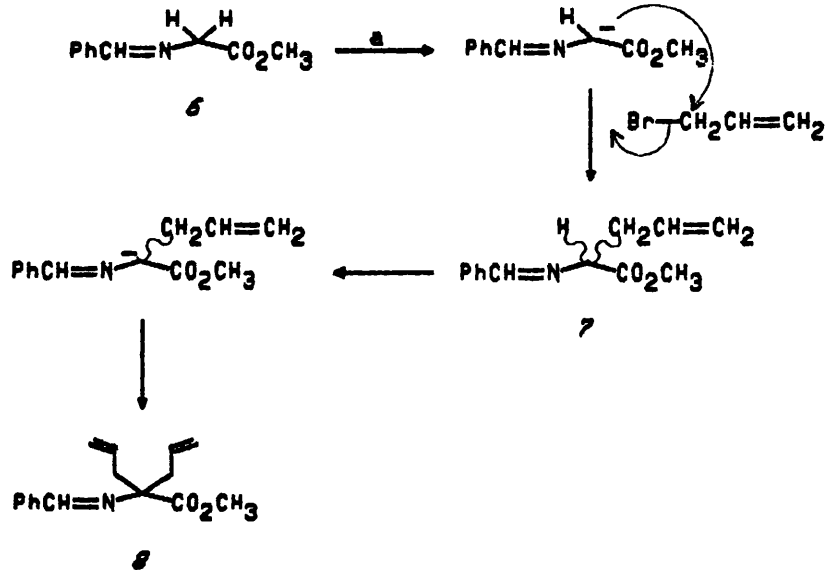
Initially the alkylated glycine (7), was synthesised

6

a:- DCM, PhCHO, TEA, RT, 48h.

Scheme 8.6

in poor yield (*ca* 40%) due to a second alkylation reaction, in which the glycine residue is di-alkylated to give (*8*) (Scheme 8.7). Evidence for this comes from NMR data, which



Scheme 8.7

shows two OCH_3 peaks at 3.85 and 3.88ppm for the mono- and di-alkylated products respectively. The ratio of mono-:di-alkylated product was calculated by the ratio of the integral peak heights of the α-CH of the mono-alkylated product (*7*) to that of the combined integral peak height due to the two OCH_3 groups. Initially, the yield of (*7*) was

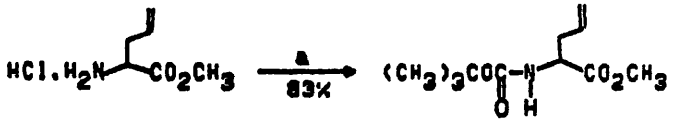
40%, isolated as the hydrochloride salt, but this was increased considerably to 60% under the reaction conditions used, as described in the EXPERIMENTAL section.

The presence of (*8*) was also confirmed by MS data [m/e (C.I.); $(M+1)^+$, 170; hydrochloride salt]. The two products were not separated as it was considered that (*8*) would react very much more slowly than (*7*), due to the greater steric hinderance, as a result of the two alkyl-side chains.

The alkylated glycine could be de-protected by one of two methods:- i) by bubbling HCl gas into an aqueous methanolic solution of the product, followed by removal of the methanol *in vacuo*, before washing with ether, and removal of the water *in vacuo* or ii) by vigorously stirring a solution of the product, dissolved in 2M HCl, with ether for one hour at ambient temperature, before washing with ether, and subsequent removal of the aqueous acid *in vacuo*.

8.3.2 Synthesis of tert-butyloxycarbonyl-D,L-(allyl)glycine methyl ester (*9*).

The synthesis of this compound (*9*), was achieved by using a slightly modified method to that used for the synthesis of (*1*) (Scheme 8.8). Considerably more base is used to ensure that any remaining acid from the previous step was neutralised. A slightly larger quantity of $(Boc)_2O$ was also used to ensure complete reaction of the starting material. As proposed earlier, the rate of reaction of the di-alkylated (*8*), compared to that of the mono-alkylated



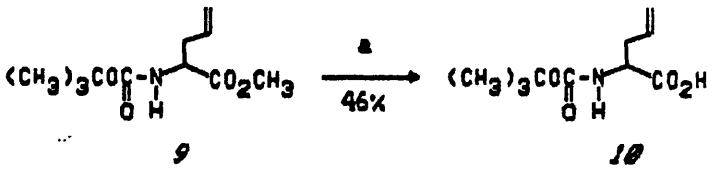
a:- dioxane/H_2O, Na_2CO_3, $(Boc)_2O$, 24h, 0^0C-->RT.

Scheme 8.8

(7) glycine residue, would be slower. This has been confirmed by NMR measurements on *(9)*, which shows only one peak due to OCH_3 at 3.74ppm. The absence of *(8)* is also shown by the greater simplicity of the NMR spectrum.

8.3 Hydrolysis of tert-butyloxycarbonyl-D,L-(allyl)glycine methyl ester *(10)*.

Hydrolysis of *(9)* to the free carboxylic acid *(10)* (Scheme 8.9), was affected using aqueous NaOH, a method based on that of Iselin et al.[31] Hydrolysis of alkyl esters



a:- MeOH, NaOH (aq), 0^0C, 3h.

Scheme 8.9

of peptides with alkali is a frequently used method for the removal of blocking groups from the C-terminal carbonyl group, but the method is far from being specific, especially if the peptide chain is long, where a danger of hydantoin formation exists (Fig 8.20). If found, the hydantoin could ring open under the influence of excess alkali with the production of a urea derivative. This ring-closure-elimination reaction[32] occurs particularly readily if glycine is the second amino acid in the sequence.[33] Fortunately hydantoin formation only appears to occur if the carbonyl group in urethanes is flanked on both sides by

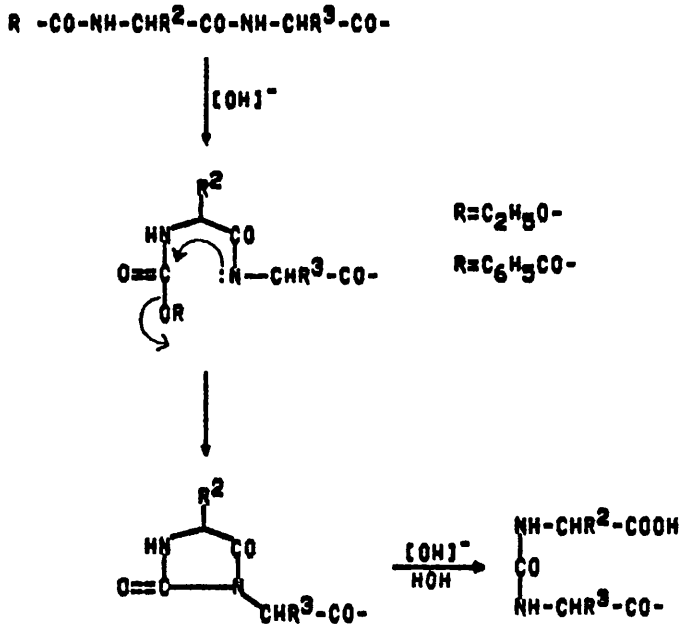


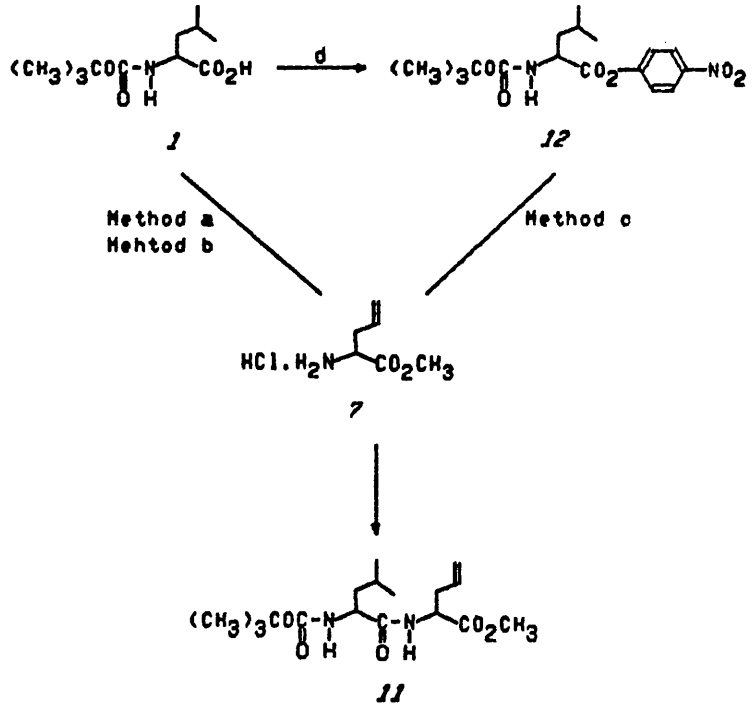
Fig 8.20

atoms with unshared pairs of electrons, as illustrated above in Fig 8.20. Most side reactions can be kept to a minimum by performing the saponification at 0°C rather than at room temperature, by adding the alkali in small

portions, and avoiding excess. However, methyl ester peptides are useful as they are easily converted to the corresponding hydrazide.

8.4 Synthesis of tert-butyloxycarbonyl-L-leucine-D,L-(allyl)glycine methyl ester (11).

The synthesis of this compound (11), was achieved by three methods:- a mixed anhydride method using IBC, b activated esters, and c acid azide method (Scheme 8.10).



a:- DMF, (7)/base, IBC, -20^{0}C-->RT; b:-DMF, (7)/base, DPPA, RT; c:- DMF, (7)/base, HOBt, RT; d:- EtOAc, DCCI, p-NP, 0^0-->RT.

Scheme 8.10

8.4.1 Mixed anhydride method.

The synthesis of the di-peptide *(11)*, was successfully achieved in 49% yield. The methodology is similar to that used for the synthesis of the oligo-leucine peptides (3), (4) and (5), the mechanism of which has already been discussed.

The lower yield in comparison to the synthesis of the di-leucine peptide (3), 49% compared to 80%, may be due to steric factors (Fig 8.21). The carbons at C1 and C2 are both electrophilic, but C2 is less so, due to the electron releasing nature of the alkoxy-group. It would therefore be

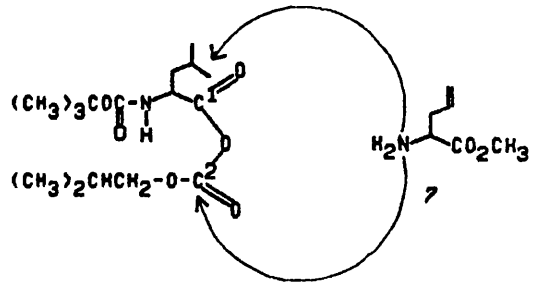


Fig 8.21

reasonable to assume that the amino component is more likely to attack the "correct" carbonyl group. However, because the alkenyl-glycine residue (7) is less sterically hindered than the corresponding leucine residue (2), it may be possible for amino component to attack at C2 as well as at C1 even though C2 is less electrophilic. (Fig 8.21). Initially this method for synthesising the di-peptide was unsuccessful, as the amino component attacked the "wrong" carbonyl carbon centre, to form instead a urethane type

compound (Fig 8.22). The formation of this product was unexpected, as it had been reported by Alberston,[35] that neutral amino acids that had been modified eg., halogenated, nitrated, unsaturated, could be employed with

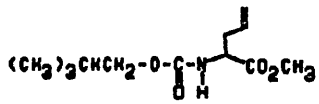


Fig 8.22

the mixed anhydride method. Two explanations seem possible for the formation of the urethane by-product:- i) as already mentioned, attack at the "wrong" carbonyl centre after the formation of the mixed anhydride or ii) attack of the amino component on the un-reacted IBC, which had failed to form the mixed anhydride (Fig 8.23). Extensive urethane formation has however, been reported by Alberston[35] in the coupling of the IBC mixed anhydride of 2-acetamidononanoic acid with amines, and in the attempted coupling of mixed anhydrides of pyruvic acid with esters of amino acids. In the latter case, a cyclic intermediate has been inferred, which altered the point of attack of the amino ester from which would normally be expected (Fig 8.24). Subsequent syntheses of the di-peptide using this method did not produce any urethane by-product detectable by either TLC analysis or by ^{1}H NMR spectroscopy on the crude reaction mixture.

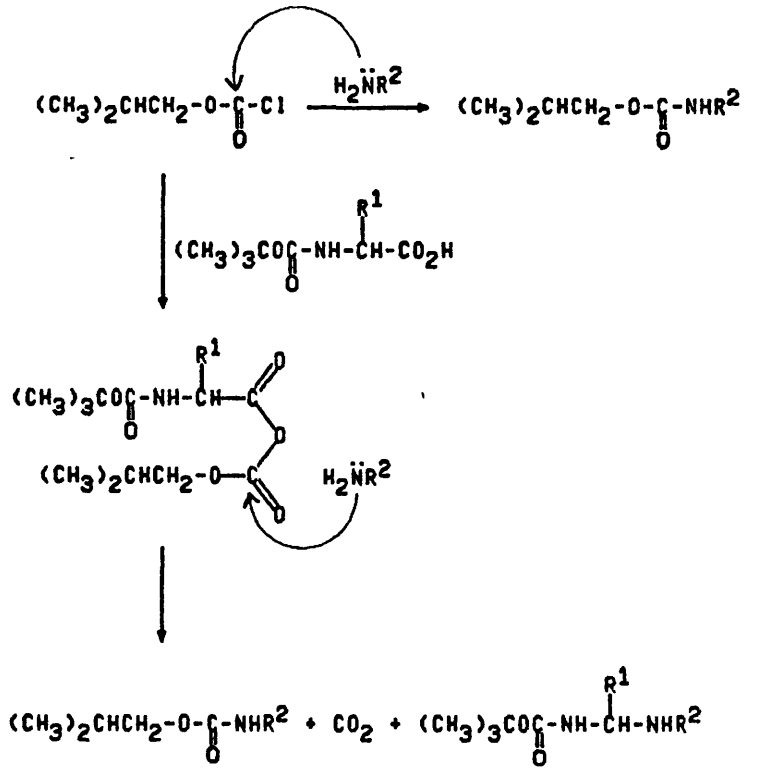


Fig 8.23

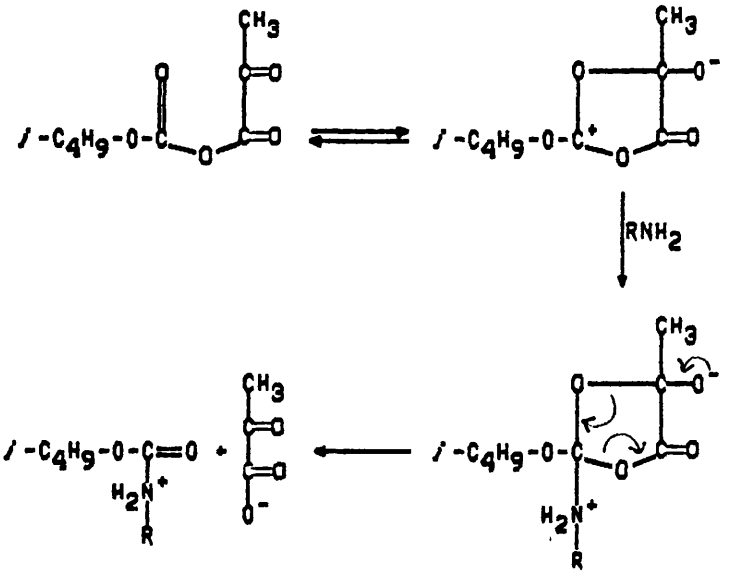


Fig 8.24

8.4.2 Activated ester method.

Unequivocal coupling reactions can only be achieved if a single electrophilic centre is present in the acylating agent, and thus the nucleophile, the amino component, is acylated in a unique way. The inherent presence of two electrophilic groups in mixed anhydrides suggests that they cannot entirely fulfil this requirement of a "ideal" acylating agent, although mixed anhydrides obtained with phosphoric acid may be exceptional in this respect. Symmetrical anhydrides of protected amino acids also have two reactive carbonyl groups, but because of the symmetry of the molecule, the two acylation products are the same. An alternative addition to unequivocal acylation is if the electron with-drawing substituent used for the activation of the carbonyl-group were to play the role of an acylating agent, then only the amino acid carbonyl can become part of the newly-formed peptide bond. Such substituents include acid chlorides, and azides, which, although prone to other side reactions, do not form second acylation products since the leaving group cannot form amides.

Bodansky was prompted to re-consider the relationship between the electron distribution in thiophenyl esters, originally proposed by Wieland *et al*,[36] after Gordon, Miller and Day[37,38] observed that the ammonolysis rates of vinyl and phenyl esters exceeded those obtained with alkyl esters. The comments of Gordon, Miller and Day[37,38] suggested that thiophenyl esters of protected amino acids owed part of their reactivity to the fact that they are esters of thio-

esters, and that their ability to form amides under mild conditions was probably due to the fact that they are acyl esters (Fig 8.25). Bodansky[40] examined the negatively

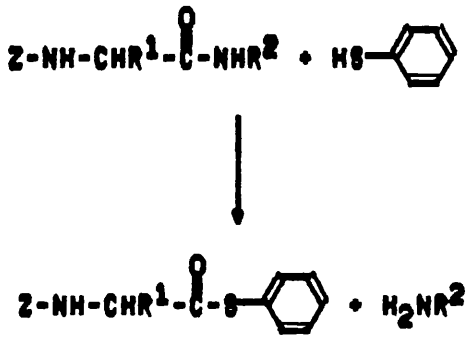


Fig 8.25

substituted phenols *ortho*-, *meta*-, *para*-nitrophenols, and 2,4-dinitrophenol. However, esters of the latter were too reactive, and accordingly too sensitive to hydrolysis by water in the reaction mixture. Of the mono-substituted phenols, the *para*-derivative was selected as the main-stay of peptide synthesis, due mainly to the readiness of its esters to crystallise. Some advantages of the *ortho*-nitro-analogs were overlooked, and were not recognised until many years later,[41-43] and *para*-nitrophenyl esters of benzyloxycarbonyl-amino acids were used in practical synthesis,[44,45] as demonstrated by the synthesis of oxytocin.

The synthesis of the *para*-nitrophenol ester derivative (12) was achieved in 60% yield. *para*-Nitrophenol was re-crystallised from toluene before use, and the product prepared with careful exclusion of moisture due to the ease with which the ester hydrolyses when in solution. The

reaction vessel was also covered if aluminium foil in order to minimise the possibility of photo-decomposition of the product formed in the reaction mixture.

The di-peptide (11), was synthesised in 38% yield. The low yield obtained is attributed to a poor batch of the mono-alkylated glycine. The addition of 1-hydroxy-benzotriazole (HOBt) is claimed to catalyse acylation,[48] and to suppress racemisation, although there has been some doubt about the latter. The mechanism for its catalytic action is a little unclear, but it has been suggested that the HOBt displaces the ester functional group, protecting the carbonyl group of the amino acid ester, to from a more reactive HOBt ester intermediate. Supporting evidence for this postulate comes from the isolation of a crystalline compound[5] (Fig 8.26), bands in the IR spectrum of which are

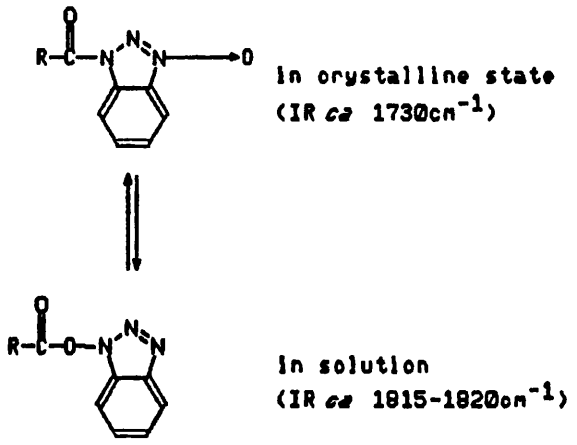


Fig 8.26

consistent with A (solid state, 1730cm^{-1}), and in solution with B (1815-1820cm^{-1}). The displacement of the para-nitrophenyl ester can be written as illustrated in (Fig

8.27). Subsequent nucleophilic attack by the amino acid component gives the peptide, and also re-generates the catalyst (Fig 8.27).

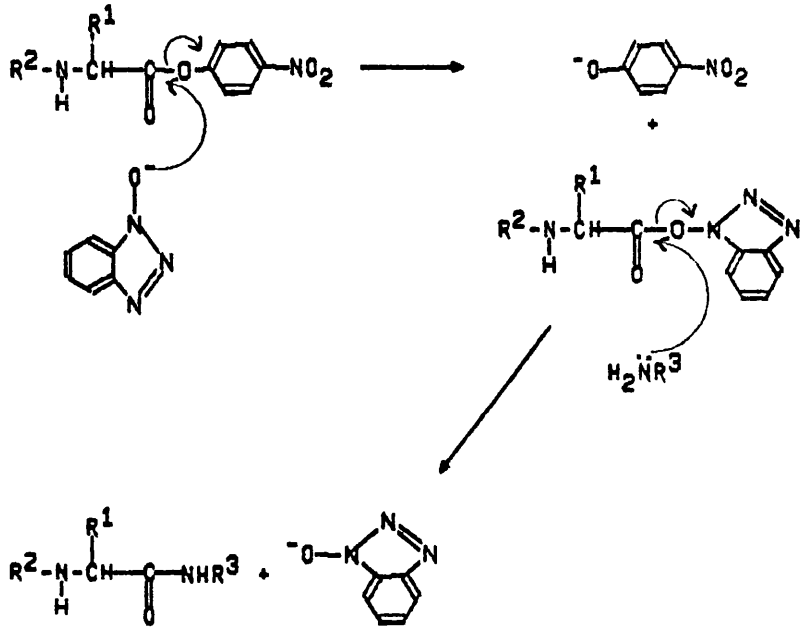


Fig 8.27

The additive HOBt, is usually employed in an equimolar amount with the two components to be coupled, hence there are two moles of nucleophile present in the reaction mixture for each mole of carbonyl component. Therefore, the life-time of highly reactive reactive intermediates is considerably reduced. The concentration of the additive, which is acting as a second nucleophile, hardly changes during the coupling reaction, because it is continually being re-generated. Hence there is a significant change in the kinetics of the process. The active ester, formed *in situ*, is less conducive to side reactions, and yet it is sufficiently reactive to ensure satisfactory reaction

rates.

8.4.3 Acid azide method.

Synthesis of the di-peptide (11), was achieved in 61% yield using diphenylphosporylazide (DPPA) to form the azide *in situ*.[49] The DPPA reagent converts peptides with a free carboxyl group at the C-terminus to the desired acid azide without the need to isolate intermediates. The mechanism of this reaction was elucidated by Shioira *et al*[50] (Fig 8.28).

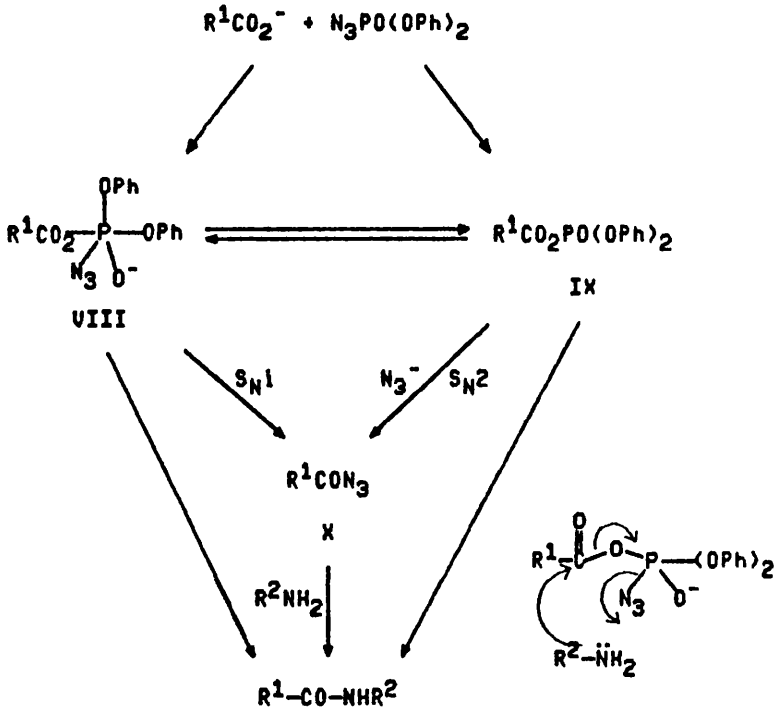


Fig 8.28

The carboxylate anion may attack the phosphorus atom of DPPA to give the penta-valent compound (VIII), and the mixed anhydride (IX). An equilibrium exists between (VIII), and (IX). The amino component will attack (VIII), and (IX)

to give the amide. Spectroscopic studies of racemisation during the Young test[23] were also performed by Shioira et al.[50] They found no oxazolone formation, by observation of the IR spectrum at 1830cm^{-1} when an equimolar mixture of benzoyl-L-leucine and TEA in chloroform was treated with an equimolar DPPA at 0°C. However, when the Young test was performed after the formation of (IX), by the action of diphenylphosphorochloridate with benzoyl-L-leucine in the presence of TEA using the Zervas procedure,[51] both the yield and optical purity were inferior to that of the DPPA method above. This suggests that direct attack of the amino component to (IX) will occur to a lesser extent. The intramolecular migration of the azido-group of (VIII), from the phosphorus to the carbonyl carbon by an S_Ni type re-arrangement, will give the carboxylic acid azide (X), which may also be formed from (IX), by an S_N2 type reaction of the azide anion with (IX). The intermediacy of the acyl-azide (X) was demonstrated by the detection, by TLC, of benzazide, when benzoic acid was condensed with n-butylamine using a mixture of DPPA and TEA. The acyl-azide (X) is an intermediate of the azide method of Curtius,[52] which is thought to be essentially free of racemisation, but it is now known that a loss of optical purity, particularly in the presence of excess base,[53] can occur.

8.4.4 Conclusion.

The low yield from method b is attributed to a poor batch of the mono-alkylated glycine, in which the ratio of

mono-:di-alkylated glycine may have been less than 1:1. In any event, the extra step required to synthesise the para-nitrophenyl ester intermediate (*12*) more or less prohibited the use of this method, based on a yield consideration alone, in which the intermediate was isolated in 60% yield, although yields of up to 75% have been reported[54] for the synthesis of para-nitrophenyl esters. The esters are also

Table 8.2. Comparison of yields, and elemental analyses of the di-peptide (*11*) from the three methods above (8.4.1-8.4.3).

Method	Yield/%	Found elemental analyses[a] (C, H, N)%
a) Mixed anhydride	49	59.8, 9.06, 7.98
b) Activated esters	38	60.2, 9.06, 7.89
c) Acid azide	61	59.6, 9.11, 8.19

a:- $C_{17}H_{30}N_2O_5$ requires:- C, 59.7; H, 8.77; N, 8.19%.

very easily hydrolysed when in solution, so all solvents must be rigorously dried in order to obtain the maximum yield of the ester product. The product, once isolated in its crystalline state, can be stored, sealed in a dark container in order to minimise photo-decomposition.

The mixed anhydride, <u>method a</u>, gave yields that were less than expected. This may be because reaction is also favoured at the "wrong" carbonyl group due to the fact that

the attacking amino component may be less sterically hindered than the corresponding leucine derivative, and so provides a greater opportunity for attack at the "wrong" carbonyl group, to form the urethane by-product, although none was detected from the reaction mixture.

Of the methods employed, method c was considered to be the best, based solely on a yield consideration alone. However, the product *(11)* was formed in the presence of a number of unidentified side products, but this may have resulted from the use of a mixture of mono- and di-alkylated glycines. It may therefore be beneficial if, in future, the mono- and di-alkylated glycine products were separated, and isolated before proceeding to the next stage of the synthesis.

The data obtained on the product *(11)* from all three methods confirms its identity. The MS spectra of the product from the above methods gave a molecular ion peak [342 (M^+) or 343 $(M+1)^+$], with the characteristic loss of the protecting group [$(CH_3)_3COC(O)$; (M^+-101)]. The ^1H NMR spectrum of the products were very similar, with few differences in the shape or position of the peaks, and elemental analysis of the products is in good agreement with that calculated for the required product. The product was also assayed using HPLC, but without conclusive results, for although the chromatogram suggests the possibility of two peaks, they were not well enough separated to be positively identified.

8.5 De-protection of tert-butyloxycarbonyl-L-leucine-D,L-(allyl)glycine methyl ester.

De-protection of (11) was performed in a similar manner to the de-protection of the oligo-leucine peptides, except that anisole was added as an electrophile trap (Fig 8.29). This minimises/prevents electrophilic attack by any free protons on the terminal double bond of the alkylated glycine residue forming a carbonium ion, and possible subsequent polymerisation (Fig 8.30). While anisole was

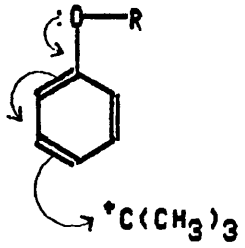


Fig 8.29

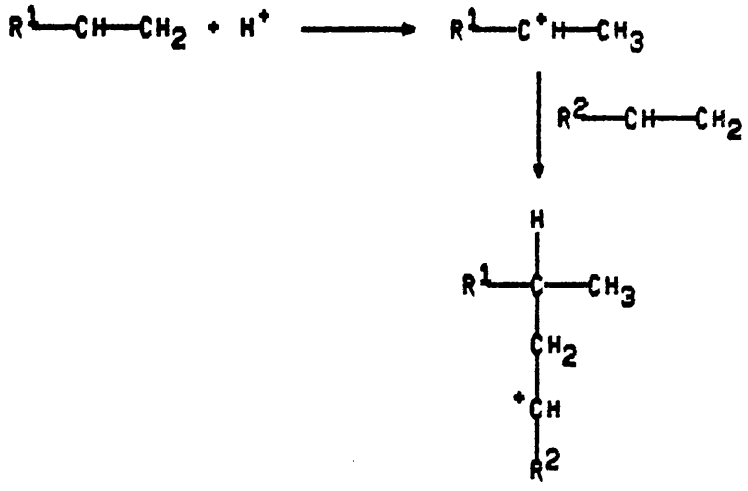
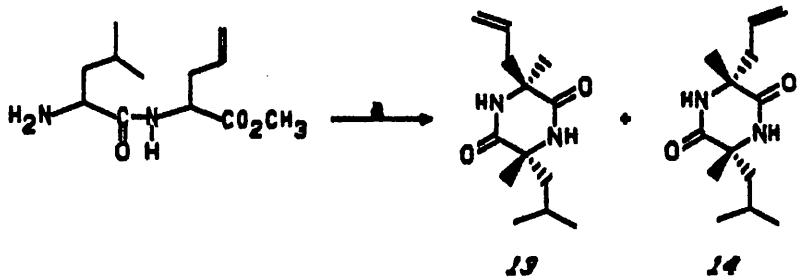


Fig 8.30

found to fulfil the purpose of de-protection in this step, it is not considered as an ideal "scavenger" as it can participate in electrophilic aromatic substitution with carbonyl groups in amino acid side chains, or the C-terminal α-carbonyl group. It can also be the source of methylating agents, which in turn can alkylate sensitive amino acid side chains, such as the thioether sulphur in methionine.[55] The de-protected di-peptide was used without purification or characterisation.

8.6 Synthesis of cyclo-L-leucine-D,L-(allyl)glycine (13) and (14).

The de-protected di-peptide (*11*) was cyclised (Scheme 8.11) using the method of Nitechi *et al.*[7] The diastereomers were successfully separated by column chromatography using silica gel. The two individual diastereomers [isomer-1 (*13*) and isomer-2 (*14*)] were isolated from an approximate 1:1 mixture (1.95g and 1.83g; total 3.78g, 86%).



13 *14*

a:- toluene/butan-2-ol, reflux, 48h.

Scheme 8.11

Using the TLC solvent system [chloroform-methanol-acetic acid (14:2:1)], and developing in $KMnO_4$ solution, the configuration of the diastereomers was provisionally assigned, since using the above solvent system, the *anti*-isomer has been shown by Nitechi et al[7], in their study of a simple route to sterically pure diketopiperazines, to run consistently ahead of the *syn*-isomer. The R_f values of the two isomers in solvent system were 0.74 [anti-isomer {isomer-1} (*13*)], and 0.71 [*syn*-isomer {isomer-2} (*14*)].

The ^1H NMR, taken in D_6 DMSO shows clear differences between the two isomers-1 and 2. The peaks, δ=0.87ppm [$(CH_3)_2$, leu] (C10, Fig 8.31), are a set of quartet, or a doublet of doublets (*J*=6.8Hz) for isomer-1, and a set of triplets (*J*=6.6Hz) for isomer-2. In the spectrum of one isomer, the expected pairs of doublets appears as a triplet due to the overlap of the inner lines, and in the other, as four evenly spread lines since $J_1=J_2=\frac{1}{2}\delta_1-\delta_2$. The set of peaks

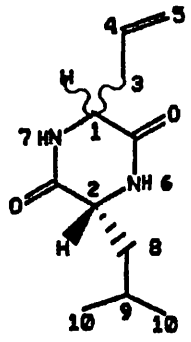


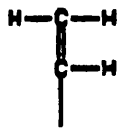
Fig 8.31

due to C8 appears as a triplet (δ=1.54, *J*=6.8Hz) for isomer-1, but as a pair of identical multiplets (centred at

δ=1.45 and 1.60) for isomer-2. The protons at C3 resonate as a pair of identical multiplets (centred at δ= 2.39 and 2.56) for isomer-1, but as a broad multiplet (δ=2.44) for isomer-2. The peaks from C2 (δ=3.70 and 3.76) and C1 (δ=3.97 and 3.91), the chiral centres, are well defined in the spectrum for isomer-1, but merge into one another in the case of isomer-2. There is variation in the coupling constants of C5 and C4. In isomer-1, both the *trans* and *cis* coupling constants are greater by *ca* 3-5Hz (Table 8.3).

Nuclear Overhauser experiments were performed on both isomers-1 and 2 in an attempt to assign configurations to the two diastereomers, but they were inconclusive.

Table 8.3. *Trans* and *cis* coupling constants (Hz) of the terminal double bond for isomer-1, and isomer-2.



Isomer	trans	cis
1	13.2	8.9
2	10.1	6.9

The problem was resolved by performing lanthanide shift studies on both isomers using $Eu(fod)_3$ (Fig 8.32) as the shift reagent, the results of which are tabulated

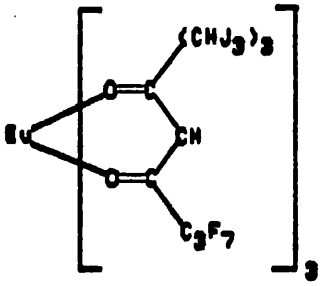


Fig 8.32

(Table 8.4). The data listed in the table indicate a reasonable linearity for both isomers in the concentration range used in the experiment. Isomer-1 experienced stronger paramagnetic induced shifts at H-4, 6, 8 and 9, and to a lesser extent at H-1 and 3, which indicates stronger binding to the lanthanide atom, and so a more stable lanthanide complex is formed.[NB 1] The paramagnetic induced shifts for isomer-1 are strongest at H-4, 6, 7, 8 and 9. Complexes formed between the lanthanide shift reagent, and both isomers are probably of the type illustrated in Fig 8.33. In the possible complexes formed by isomer-1, the lanthanide atom may approach the ring from either side (Fig 8.34AB). For isomer-2, only approach form one side is

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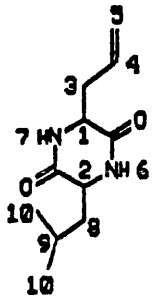
NB 1 The ability of Eu to change chemical shift because of the paramagnetism associated with its unpaired electrons is a through space effect, which falls off inversely with the cube of the distance between the Eu atom and the nucleus concerned. This has been named pseudo-contact to distinguish it from the so-called true contact shift. This latter term is used for an association phenomemon in radical chemistry, in which direct coupling between the unpaired electron of a radical and the nuclear spin causes changes in NMR resonance frequency of the nucleus suffering the coupling. Although the preferred generic term is shift reagent, these compounds are often named lanthanide shift or contact shift reagents.

Table 8.4. Lanthanide shift studies of cyclo-L-Leu-D,L-(allyl)glycine (13 and 14).



	Chemical shift (ppm)									
$Eu(fod)_3$a	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8	H-9	H-10
Isomer-1										
1) 0	4.03	3.96	2.65	5.73	5.25	6.48	6.21	1.68	1.78	0.97
90.4	5.40	5.12	3.56	6.37	5.54	7.89	7.27	2.58	2.26	1.19
245.5	5.88	4.86	3.35	7.56	6.03	10.28	9.40	4.57	4.17	1.59
2) 482.3	6.15	5.91	5.27	10.47	6.65	13.23	12.22	8.25	8.81	2.08
$\Delta\delta_H$b	2.12	1.95	2.62	4.74	1.40	6.75	6.01	6.57	7.03	1.11
Intercept	4.59	4.28	2.71	5.50	5.28	6.61	6.21	1.44	1.20	0.99
Slope	3.80	3.34	4.88	9.84	2.90	13.99	12.57	13.81	14.94	2.31
Isomer-2										
1) 0	4.05	3.97	2.64	5.76	5.24	6,72	6.47	1.84	1.65	0.98

81.0	4.82	4.82	3.18	6.15	5.46	7.53	7.38	2.51	2.23	1.15
313.7	4.73	4.73	3.47	7.10	5.99	10.51	9,82	4.33	4.33	1.65
2) 468.9	6.11	6.11	4.31	7.92	6.32	11.24	8.47	5.36	5.65	2.23
$\Delta\delta_H{}^b$	2.06	2.14	1.67	4.16	1.08	4.52	2.00	3.52	4.00	1.23
Intercept	4.17	4.13	2.73	5.76	5.26	6.81	6.93	1.88	1.60	0.94
Slope	3.50	3.62	3.11	4.51	2.29	10.15	5.14	7.54	8.64	2.59

a:- moles $Eu(fod)_3$/moles substrate x10-3; b:-$\Delta\delta_{Hb}$=1)-2)

possible to give the complex, as shown in Fig 8.34C. Both complexes A and B of isomer-1 (Fig 8.33) are expected to be present in solution. Of particular note is the extent to which H-7, 8 and 9 of isomer-1 are affected by the shift reagent in comparison to isomer-2.

From the data in Table 8.4 $\Delta\delta_H$ for H10 changes very little for either isomer, indicating that the two methyl groups of the leucine residue do not lie over the plane of the ring, perhaps due to steric hindrance. H-8 and 9 change quite dramatically for isomer-1, because the lanthanide atom is able to approach the plane of the ring such that the induced paramagnetic effect is experienced much more strongly by these protons.

It may be possible that the alkenyl chain of either isomer to lie in such a way as to lie over the plane of the ring (Fig 8.35). This might also be possible for the leucine residue, if the two methyl groups face away from

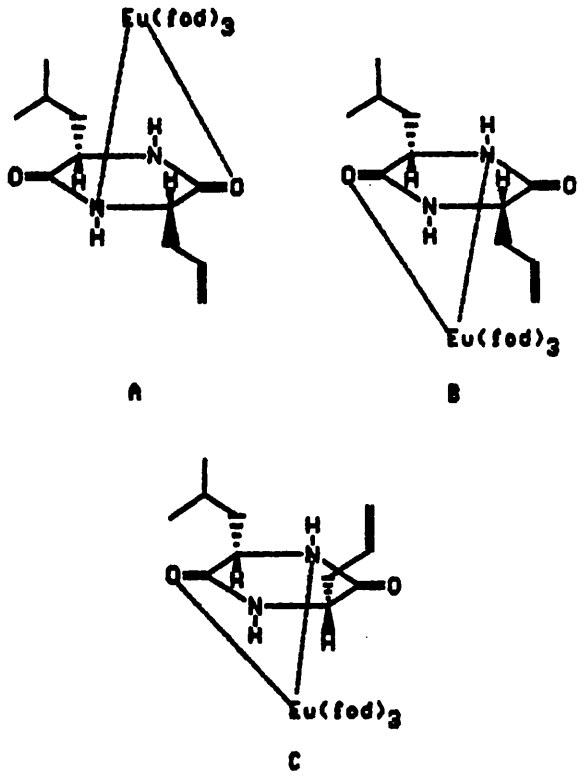


Fig 8.33

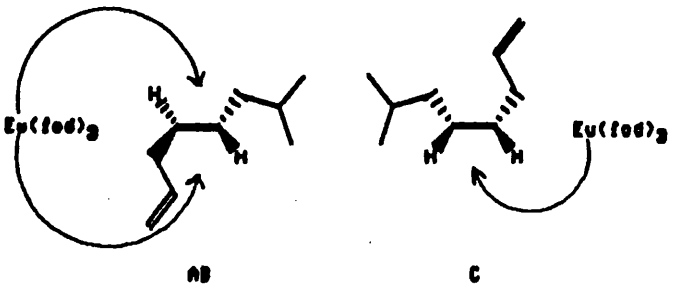


Fig 8.34

the plane of the ring (Fig 8.36). For isomer-2, steric hindrance will ensure that only the leucine or the alkenyl side chain will lie over the plane of the ring.

The protons H-1 and 2, the αCH protons, of isomer-1, experience changes in chemical shift, but never coincide throughout the experiment, unlike those of isomer-2, which

do become co-incident, remaining so with increasing concentration of shift reagent. This indicates that these protons of isomer-1 remain in different magnetic environments, ie., the protons are on opposite sides of the

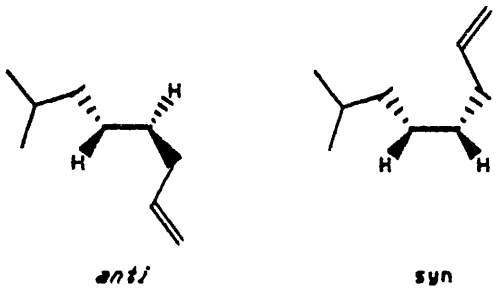


Fig 8.35

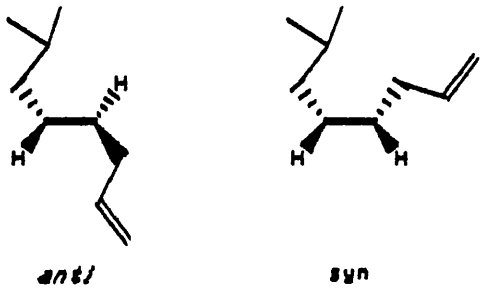


Fig 8.36

plane of the ring, and that the protons in isomer-2 remain in a similar magnetic environment throughout the experiment (Fig 8.33C and Fig 8.34C). This supports the view that the protons of isomer-1 are on opposite side of the plane of the ring (Fig 8.33A/B and Fig 8.34A/B).

H-3 and 4 show a much smaller change in $\Delta\delta_H$, however, the difference between isomer-1 and isomer-2 may suggest that the distance between the lanthanide atom and H-3 is

closer in isomer-1 than in isomer-2. On the basis of the above argument, the configurations of the two diastereomers have been assigned as:- isomer-1 has been asigned the *anti*-configuration (*13*), and isomer-2 the *syn*-configuration (*14*).

Further supporting evidence comes form the measurement of the optical rotations of each diastereomer, in which the *anti*-isomer gave a measured rotation of $[\alpha]_D$= +13°, and the *syn*-isomer $[\alpha]_D$= -53.9°.

A blocky crystal of cyclo-L-leucine-D-(allyl)glycine (*13*), of approximate dimensions 0.35x0.4mm, was produced in order to perform X-ray crystallographic studies, and confirm the above findings.

Crystal data:- $C_{11}H_{18}O_2N_2$, M=210.26, monoclinic, a=7.435(2), b=7.559(5), c=10.109(3)Å, b=107.992(2), U=576.7Å, space group $P2_1$, Z=2, D_c=1.37gcm^{-3}, μ=(Mo-K_α)=0.49cm^{-1}, F,(000)=228. Data were measured at room temperature on a Hilger and Watts Y290 four-circle diffractometer in the range $2<\theta<22°$. 1537 reflection were collected of which 1213 were unique with $I \geq 3\sigma(I)$. Data were corrected for Lorentz and polarisation effects and for absorption.[2] The structure was solved by conventional direct methods.[3,4] All atoms were allowed to vibrate

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1. N. Walker and D Stuart; DIFABS, a program to correct for absorption effects in crystals, Acta. Cryst., 1983,A39,158.

2. G.M. Sheldrick; SHELX86, a computer program for crystal structure determination, uinversity of Goetingen, 1986.

3. G.M. Sheldrick; SHELX76, a computer program for crystal structure determination, uinversity of Cambridge, 1976.

anisotropically in the final stages of convergence. Hydrogens were included at calculated positions. Final residues after twelve cycles of full-matrix least squares were $R=R_w=0.0786$ for weight units. The total number of parameters varied was 135. Maximum final shift/esd was 0.002, and the maximum and minimum residual densities were 0.21 and $-0.20 e\text{Å}^{-3}$. Final fractional atomic co-ordinates and isotropic thermal parameters, bond lengths, and angles are given in Tables **Table 8.5** and **8.6** respectively. Tables of anisotropic temperature factors and hydrogen atom positions are available as supplementary data **Table A.1-A.5**. The asymmetric unit is shown in **Fig 8.37**, along with the labelling scheme used. The amide bonds are in a *cis* arrangement. This is because the thermodynamic stability of the six-membered ring compensates for the energy required for the trans->cis arrangement. It is possible however, that the ring may not be entirely flat, but have a slight twist. A slight twist would also release some of the torsional strain that might be present if the ring were perfectly flat.

Table 8.5. Bond lengths (Å) for cyclo-L-leucine-D-(allyl)glycine **(13)**.

O1-C1	1.241(9)	O2-C3	1.242(10)
N1-C1	1.336(10)	N1-C4	1.473(10)
N2-C2	1.462(10)	N2-C3	1.324(10)

C1-C2	1.501(12)	C2-C5	1.534(11)
C3-C4	1.501(11)	C4-C8	1.553(10)
C5-C6	1.503(13)	C6-C7	1.330(130
C8-C9	1.520(12)	C9-C10	1.523(11)
C9-C11	1.481(14)		

Table 8.6. Bond angles for cyclo-L-leucine-D-(allyl)glycine (13).

C4-N1-C1	124.7(7)	C3-N2-C2	125.1(7)
N1-C1-O1	122.2(7)	C2-C1-O1	118.6(7)
C2-C1-N1	119.2(7)	C1-C2-N2	114.9(7)
C5-C2-N2	108.7(6)	C5-C2-C1	112.5(6)
N2-C3-O2	121.7(7)	C4-C3-O2	118.3(7)
C4-C3-N2	119.7(7)	C3-C3-N1	113.2(6)
C8.C4-N1	111.4(6)	C8-C4-C3	107.7(6)
C6-C5-C2	112.5(8)	C7-C6-C5	125.0(1)
C9-C8-C4	116.4(7)	C10-C9-C8	109.3(7)
C11-C9-C8	112.3(7)	C11-C9-C10	110.2(8)

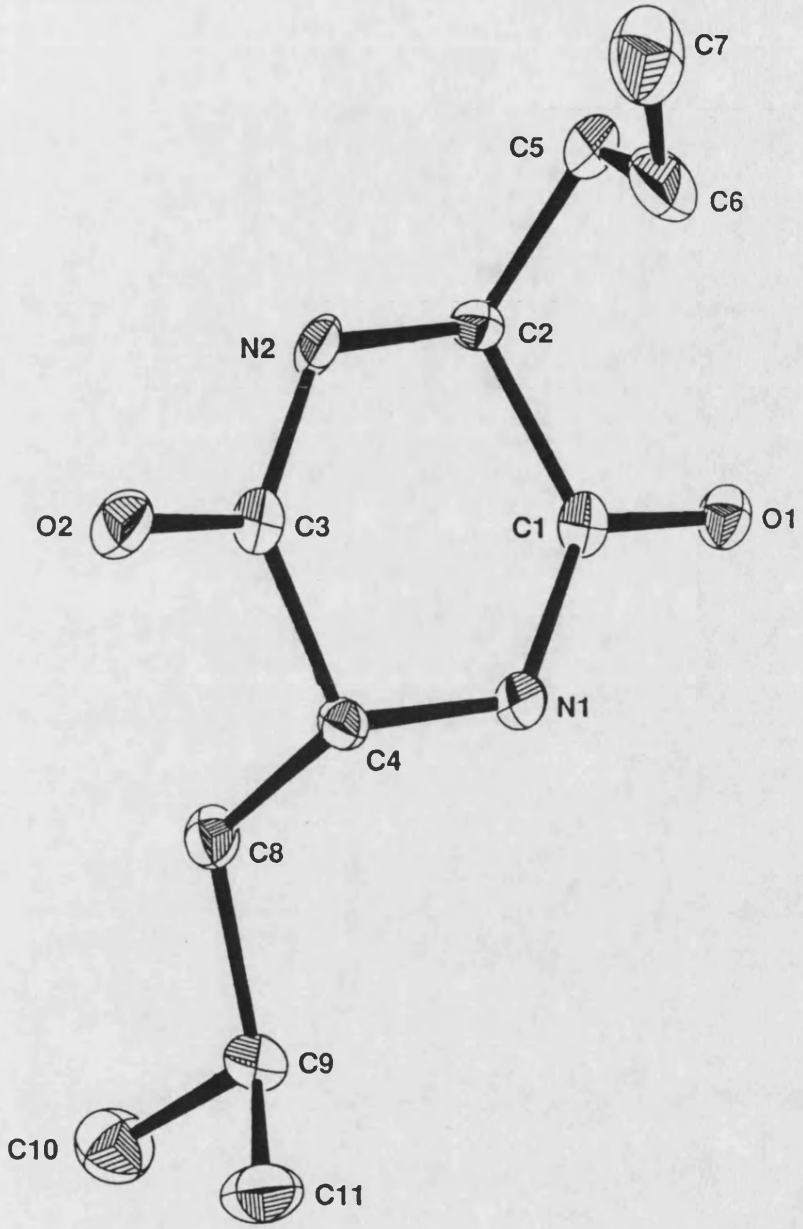
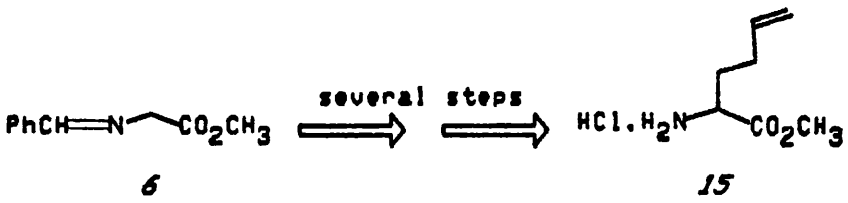


Fig 11.37. Asymmetric unit of cyclo-L-leucine-D-(allyl)glycine (*13*).

8.7 Synthesis of D,L-(pentenyl)glycine methyl ester (15).

This compound (15), was synthesised in a similar manner to that of D,L-(allyl)glycine (7), starting from the imine (6) (Scheme 8.12). No di-alkylated product was found to be present in the reaction mixture, and was confirmed by MS analysis. Assignment of the β, γ and δ protons in the alkenyl side chain in the ^{1}H NMR spectrum was attempted by performing double irradiation studies, but these were not conclusive. However, after derivatisation with a tert-butyloxycarbonyl group, the product was easier to purify and definitive assignments were possible.

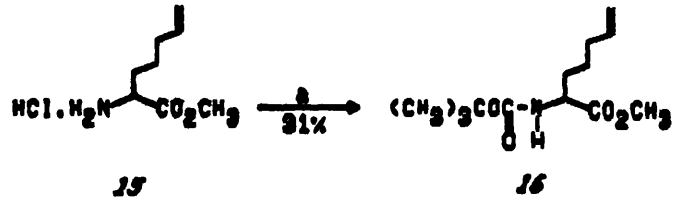


6 15

Scheme 8.12

8.7.1 Synthesis of tert-butyloxycarbonyl-D,L-(pentenyl)-glycine methyl ester (16).

The methyl ester (16), synthesised in a similar manner to that of compound (9) (Scheme 8.13). Assignment of the β, γ and δ protons in the alkenyl side chain was made with the aid of a 2D δ_H COSY spectrum. These also provided a basis for assigning the alkenyl protons of compound (15) with more certainty. It should be noted that the carefully measured line separations of the -CH= proton multiplet may



Scheme 8.13

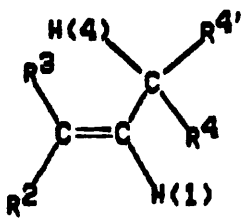
not give true *J* values, since non-first order coupling is observed. Typical values of unsaturated systems are tabulated for comparison (Table 8.7 and 8.8).

Table 8.7. H-H coupling constants (Hz) in unsaturated systems.

X	*trans*	*cis*	*gem*	Ref
(15)	18.0	9.5	1.5	
H	19.1	11.7	2.5	56
CH_3	16.81	10.02	2.08	57
CH_3CH_2	17.4	10.4	1.9	58
$(CH_3)_2CH_2$	17.3	10.4	1.6	59
$(CH_3)_3C$	17.5	10.8	1.4	60

Using a ^1H NMR computer simulation program "Raccoon",[61] it was possible to simulate approximately, the peak splitting observed in the actual spectrum (Fig 8.38, Table 8.9). Only three protons are calculated for, δ, ε and θ. Assignment of the β, γ and δ carbons from ^{13}C NMR spectrum

Table 8.8. Proton chemical shifts and spin coupling constants of some alkyl substituted olefins.[61]



				Chemical shift (ppm)[a]			
R^2	R^3	R^4	$R^{4'}$	(1)	(2)	(3)	(4)
H	H	CH_3	CH_3	5.724	4.814	4.898	(1.83)[b]
H	H	H	i-C_3H_7	5.705	4.926	4.936	(2.000)[b]
H	H	H	t-C_4H_9	5.769	4.964	4.939	1.925
H	H	t-C_4H_9	t-C_4H_9	5.738	4.98	4.779	1.565
CH_3	CH_3	t-C_4H_9	t-C_4H_9	5.208	(1.562)[c]	(1.724)[c]	1.875

				Coupling constants (Hz)					
R^2	R^3	R^4	$R^{4'}$	J(1,2)	J(1,3)	J(1,4)	J(2,3)	J(2,4)	J(3,4)
H	H	CH_3	CH_3	10.37	17.22	6.41	1.74	-1.17	-1.43
H	H	H	i-C_3H_7	10.13	17.02	7.00	2.05	-1.15	-1.43
H	H	H	t-C_4H_9	10.02	17.10	7.46	2.37	-0.94	-1.32

H	H	t-C_4H_9	t-C_4H_9	9.97	17.01	10.65	2.63	-0.10	-0.63
CH_3	CH_3	t-C_4H_9	t-C_4H_9	-1.25	-1.25	11.37	...[e]	...[e]	...[e]

a:- Spectrometer frequency=60.00Hz; b:- Assumed, not analysed; c:- Assignment not certain; d:- J(H-CH_3).
e:- Not resolved, probably less than 0.2Hz.

Table 8.9. Data for "Raccoon" simulated spectrum of tert-butyloxycarbonyl-D,L-(pentenyl)glycine methyl ester *(16)*.

Number of nuclei=5	Operating frequency=270Hz
Minimum chemical shift=4.80ppm	Maximun chemical shift=6.20ppm
Minimum intensity=0.020	Line width=0.50Hz

Chemical shifts (ppm)

-CH_2- (1)=5.00	-CH_2- (2)=5.00
CH_2= (3)=5.49	CH_2= (4)=5.51
=CH- (5)=6.00	

Coupling constants (Hz)

	2	3	4	5
J(1:y)	7.0000	1.0000	0.0001	7.0000
J(2:y)		1.0000	0.0001	7.0000

Organic Teaching Laboratories - UW Chemistry Department
Calculated NMR spectrum of tert-Boc-D/L-(pentenyl)Gly-OMe

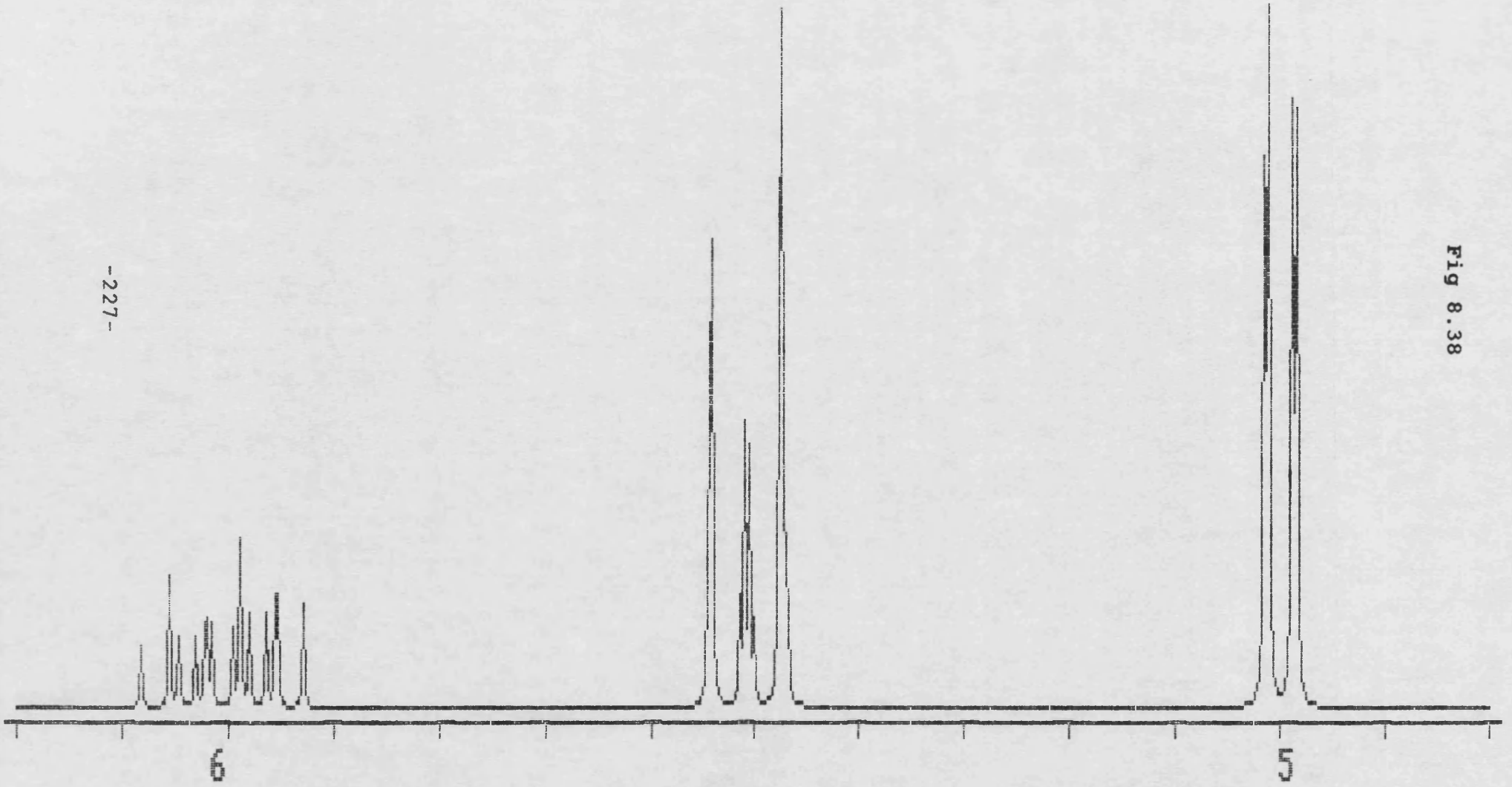
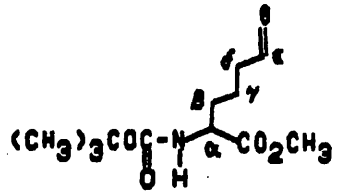


Fig 8.38

J(3:y)	1.0000	9.5000
J(4:y)		18.0000

was done by calculating their chemical shift using empirical values,[63] and subsequently comparing these calculated values with the observed values (Table 8.10). From the tabulated data, it is quite possible that the assignment of β and δ could interchange, due to the closeness of the calculated values.

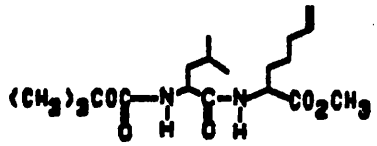
Table 8.10. Calculated and observed values of chemical shift (ppm) for the β, γ and δ carbons of *tert*-butyloxycarbonyl-D,L-(pentenyl)glycine methyl ester (*16*).



Carbon	Calculated	Observed
β	32.5	32.99
γ	24.6	24.42
δ	33.2	31.95

8.8 Synthesis of tert-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester (17).

This compound (17) (Fig 8.39) was synthesised in a similar manner to that of (11), and was isolated in 52% yield. The mechanism of this reaction has already been discussed.



17

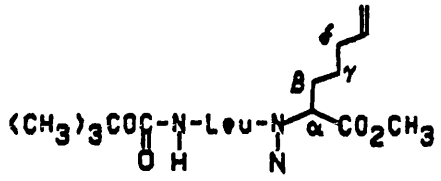
Fig 8.39

By performing a homonuclear 2D COSY experiment, it was possible to assign the protons unambiguously for both residues. The ^{13}C NMR spectrum of (17) indicates the presence of two diastereomers due to the appearence of two βCH_2 resonances. For comparison, the calculated, and observed values of the chemical shift for this compound (17) are presented (Table 8.11). The βCH_2-carbon was assigned on the basis that the αCH-carbon would show the biggest shift differences between each carbon pair of the two diastereomers.

8.9 Synthesis of cyclo-L-leucine-D,L-(pentenyl)glycine (18) and (19).

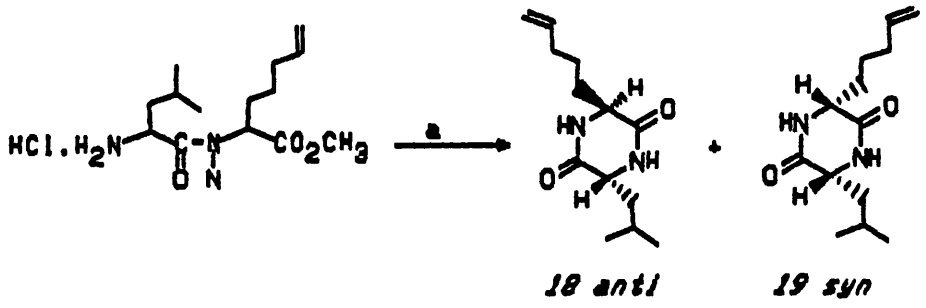
The cyclo-peptides, (18) and (19), were synthesised

Table 8.11. Calculated and observed values of chemical shift for the β, γ and δ carbons of tert-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester (*16*).



Carbon	Calculated	Observed
β	32.5	31.66 and 31.75
γ	24.6	24.36
δ	33.2	22.05

using the same method as that used for the cyclisation of (*13*) and (*14*) (Scheme 8.14).



a:- Toluene/butan-2-ol, reflux, 48h.

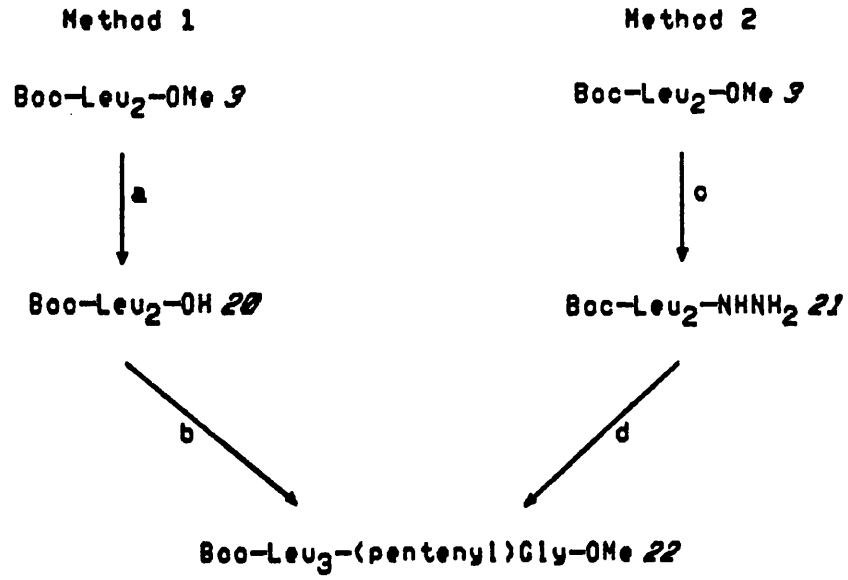
Scheme 8.14

The two diastereomers, *(18)* and *(19)*, were isolated in an approximate 1:1 ratio. NMR data was obtained for the anti-isomer *(18)*, since the syn-isomer was insoluble in the normal NMR solvents. The configuration of each isomer was assigned from optical rotation data; anti-isomer *(18)*, $[\alpha]_D$= +6.3°; syn-isomer *(19)*, $[\alpha]_D$ -35°.

In the ^1H NMR spectrum of *(18)*, taken in $CDCl_3$, the two αCH protons are almost co-incident, but more separated in D_6 DMSO. A homonuclear 2D COSY spectrum was obtained in order to identify the two near co-incident αCH protons. Correlation with other signals indicates that the αCH-Gly is at higher field than the αCH-Leu.

8.10 Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucine-D,L-(pentenyl)glycine methyl ester *(22)*.

The synthesis of this tetra-peptide *(22)*, was approached by two different methods (Scheme 8.15). Initially Method 1 was pursued, this being appearing to be the easier route to follow, on the basis of practical ease, and on past results with using IBC to form peptide bonds via mixed anhydride formation. However, the hydrolysis of the di-peptide methyl ester *(3)* to the free carboxylic acid, by the method of Iselin,[31] gave very poor yields, on several attempts. The target compound *(22)* was then approached via method 2 which involves the formation of a hydrazide intermediate. This proved to be successful, the product *(22)*, being isolated in 52% yield. The hydrazide



a:- NaOH (aq), MeOH, 0°C; b:- mixed anhydride formation via IBC + (17); c:- $NH_2NH_2.H_2O$, MeOH, RT; d:- azide formation via butylnitrite + (17).

Scheme 8.15

intermediate was isolated in yields >95% using the procedure recommended by Guttmann and Boissonnas[64] (Fig 8.40). The hydrazide was converted to the azide *in situ*, by

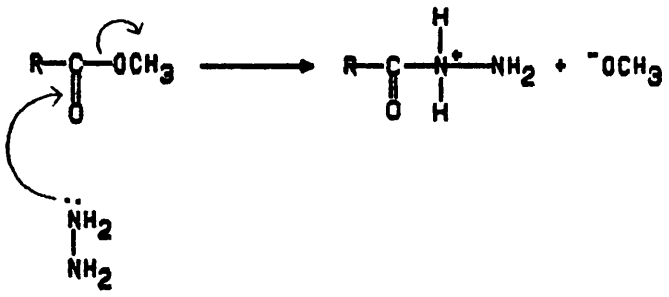


Fig 8.40

the method of Honzl and Rudinger.[65] These authors described in detail the influence on the reaction of temperature,

solvents, acidity, the type of acid, and the nitrosating agent. They concluded that preparation of the azide was best conducted at low temperature, in homogeneous solution, at high acidity, and with an organic nitrate, all of which helps suppress the formation of amide, often a problem found during the formation of the azide from the hydrazide (Fig 8.41).[68,69] In this study, the azide formed was not isloated, but reacted with the amino component after neutralisation with base. According to Honzl and Rudinger,[65]

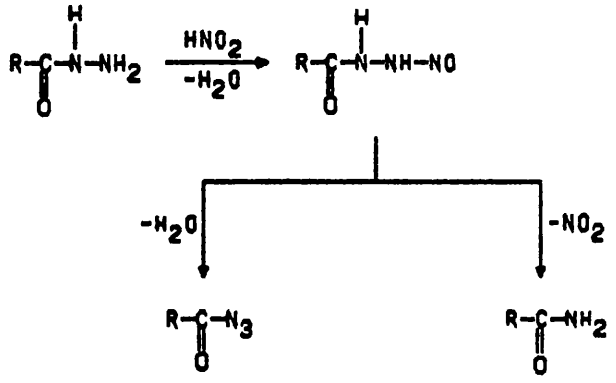


Fig 8.41

the amide is not a re-arranged product of the azide, a view supported by the observation that no amide is formed if the azide is prepared from the acid chloride, and sodium azide. The proposed mechanism involves the cleavage of the bond between the α and β nitrogens of the nitrosated hydrazide. The mechanism of azide formation is very similar to the "normal" mechanism of azide formation (Fig 8.42).

After the azide is formed, it can be acylated by the amine component, or it can produce an isocyanate, via the

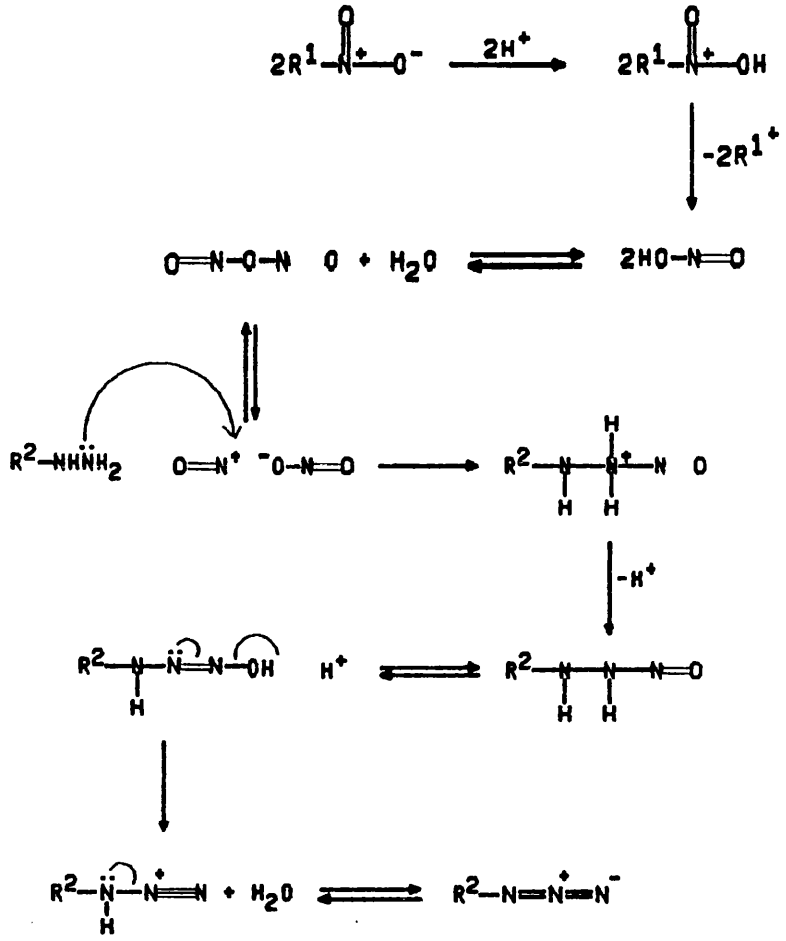


Fig 8.42

Curtius re-arrangement (Fig 8.43).[67] This is a uni-molecular process, its rate depending on both solvent and temperature, but it has also been shown to be dependant on the concentration of the reactants, including that of the azide peptide.[68,69] This is the major route in the formation of by-products, as demonstrated by the investigations of Schnabel.[70] The isocyanate can in turn react with the amino component, to yield a urea derivative (Fig 8.44).[71,72] Symmetrical ureas can also form, if the isocyanate is partially hydrolysed to an amine, which in turn reacts with

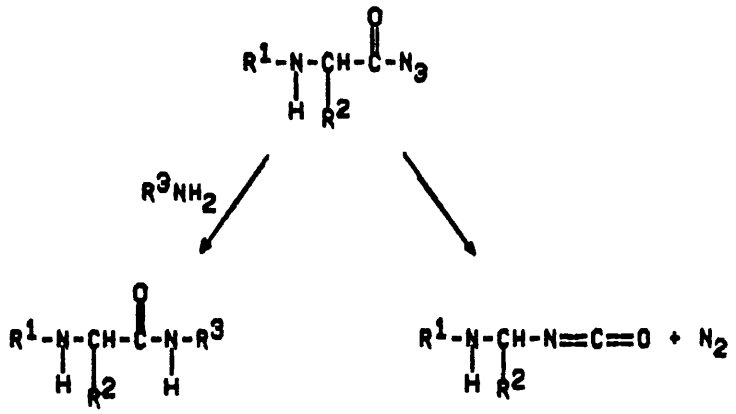


Fig 8.43

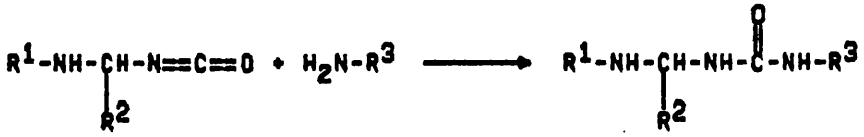


Fig 8.44

the same isocyanate. The organic azide can also acylate the still unreacted hydrazide in the reaction mixture, to form symmetrical *bis*-acyl hydrazides, especially if the conversion of the hydrazide is incomplete, or the basicity is too low (Fig 8.45).

One of the reasons for the widespread use of the azide method in peptide synthesis, is that it was thought to be racemisation free. The optical stability of acyl azides was tentatively explained by Young and co-worker.[73] They suggested that azides from which the *N*-hydrogen was abstracted by base assumed the structure shown in Fig 8.46. The oxygen of the benzyloxycarbonyl-group, instead of attacking the azide carbonyl group, is bound electrostatically

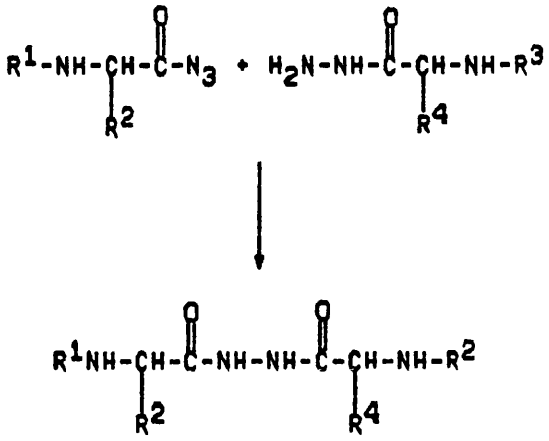


Fig 8.45

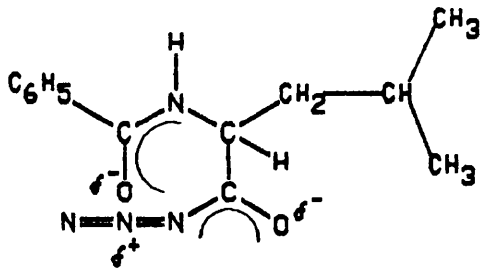


Fig 8.46

to the central nitrogen atom of the azide group. For oxazolone formation, this bond has to be rotated by 90^0, an unfavourable situation in the ring. An alternative explanation[27] may that the azides are only moderately activate, hence it is harder to abstract the *N*-proton, and are also resistant to attack by nucleophilic oxygen. Their reactivity can be explained in an analogous manner to ammonolysis of anchimerically assisted active esters.[74-76] The incoming amine forms a hydrogen bonded ring, enhancing the acylation reaction, even though the azide is not a powerful electron-withdrawing group (Fig 8.47).

However, Weygand and co-workers[77] found that azides do racemise in the presence of excess base. Anderson et al[78] isolated a racemised product (1.6% D,L), by treating Z-Gly-L-Phe-N_3 with TEA. Sieber, Brugger and Rittel,[79] observed

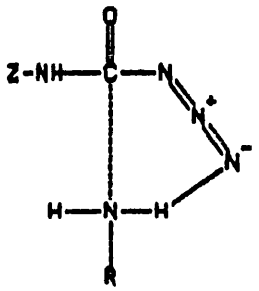


Fig 8.47

more substantial racemisation in the synthesis of a model peptide, and of Calcitonin M.[80] For example, in the coupling of Z-Val-His-N_3, 5-10% D,L-histidine was found when one equivalent of TEA or NMM was employed, and 2-3% with di-isopropylamine (DIPA). With 5 equivalents of TEA, NMM or DIPA used as base, the figures were *ca* 100, 30 and 100% racemisation respectively. In the synthesis of calcitonin M, 1-2% racemisation of histidine and phenylalanine was detected.

Kemp and co-workers[80] studied the racemisation in azide couplings, in the presence of excess base, using the isotope dilution method. The racemisation model studies used were those of Young's,[23] and Anderson's[81] racemisation models. With distilled Gly-OC_2H_5, the Anderson model yielded 0.011 and 0.03% racemate, while the Young model yielded 0.25 and 0.35% racemate. Acyl azide in DMF, with $HCl.H_2N$-

Gly-OC_2H_5, and one equivalent of TEA yielded 0.66% racemate by the Young model. With an excess of base, the Anderson model yielded 1.05% racemate. Kemp et al[80] revealed that racemisation occurs even when the azide, in order to free it from excess acids used during its formation, is isolated with dilute hydrogen carbonate solution. Substantial racemisation occurred when azides were exposed to strongly basic media. 50.3% racemisation was found with the Anderson model after 15min at 0^0C in DMF, containing 0.2M TEA. However, in practice, such excessive use of base is not required, but the results cautions against such use. Young applied his racemisation model to test azide racemisation at apparent pH9 in DMF, the azide being formed by the Honzl and Rudinger method,[65] 2% racemate was formed. The same conditions were applied for the DCCI-HOBt method of Konig and Geiger,[48] and the amount of racemate increased by 10-14%, demonstrating that while azide coupling, if properly performed, may produce a small amount of the undesired diastereomer, but this risk is far less than with the DCCI method.

8.10.1 Analysis of results.

Initially a model study was performed, based on the synthesis of oligo-tetra-L-leucine (5). The desired product was isolated in 40% yield, similar to the yield using the mixed anhydride method (41%). The synthesis of (22) was achieved in 52% yield.

The ^{1}H NMR spectrum of a diastereomeric mixture of

(22), taken in D_6 DMSO, shows two distinct sets of NH peaks due to the glycine residue (δ=8.22 and 8.25ppm). However, the intergrals are not of equal height as would be expected, indicating that one diastereomer pre-dominates slightly over the other, perhaps due to different rates of reaction. The spectrum shows three groups of αCH protons at δ=3.91, 4.23 and 4.35 for α_1CH-Leu, α_4CH-Gly, and $\alpha_{2,3}$CH-Leu respectively. The α_4CH-Gly is more complex due to accidental co-incidences. With the aid of a homonuclear 2D COSY spectrum, it was possible to assign the protons with some certainty. From the ^{13}C NMR spectrum, it was possible to observe nearly all six peaks due to the methyl group on the leucine residue, two peaks were co-incident at δ=22.84ppm. More interestingly it was possible to observe two peaks for each of the α_4CH-Gly (δ=51.44, 51.57), βCH_2 (δ=32.44, 32,57), γCH_2 (δ=30.13, 30.26) and δCH_2 (δ=24.26, 24.36) carbons. This would seem to indicate a more rigid conformation than that of compound (17), perhaps due to increased steric hindrance, resulting from an increase in the chain length by the addition of two leucine residues. TLC analysis also showed two spots, but they were too closely eluted, and no attempt was made to separate them, as a degree of cross contamination may have occurred.

8.11 Attempted cyclisation of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucine-D,L-(pentenyl)glycine methyl ester.

In the synthesis of homodectic cyclic peptides, most methods of peptide bond formation are applicable for ring closure. Nonetheless, cyclisation requires special considerations. The readiness of open chain compounds, such as linear peptides to cyclise is a function of several properties, first and foremost among these is the size of the ring to be closed. Usually no difficulties arise in the cyclisation of peptides with six or more residues, but penta-peptides are often not the best starting materials in cyclisations, and ring closure is even more hampered in most tri- and tetra-peptides. Such small rings can be closed only if at least one of the peptides bonds has a *cis* rather than the more stable *trans* geometry (Fig 8.48). The ability and readiness of di-peptides to cyclise to form diketopiperazines, in which both amide bonds are in a *cis* rather than a *trans* arrangement, has already been mentioned.

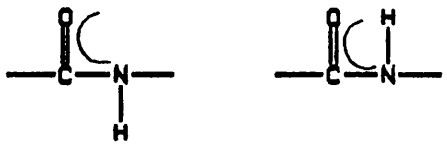


Fig 8.48

Tri-, tetra- and penta-peptides are prone to cyclo-dimerisation, in which rings of twice the expected size are

formed (Fig 8.49). This was first noted in the synthesis of gramicidin S by Schwyzer and Sieber.[82] The best explanation for this tendency for cyclo-dimerisation is the

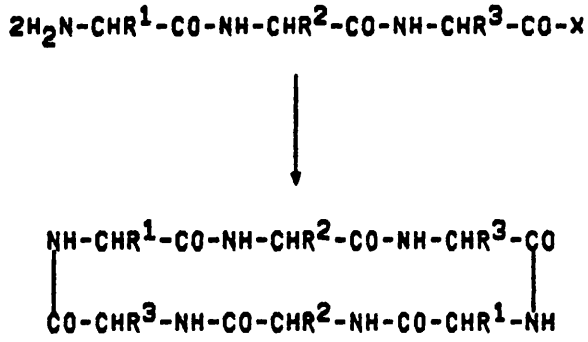


Fig 8.49

assumption of an anti-parallel arrangement of two penta-peptide derivatives prior to cyclisation. The two chains being held together by hydrogen bonds in a β-sheet like conformation (Fig 8.50). While no monomer was obtained in their early experiments,[82] in subsequent studies conditions

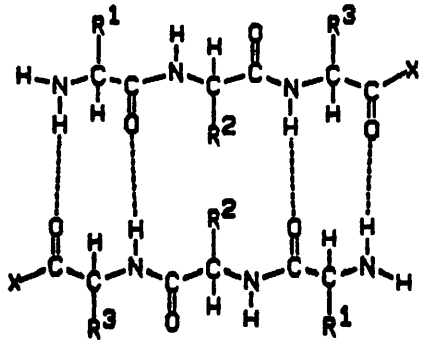


Fig 8.50

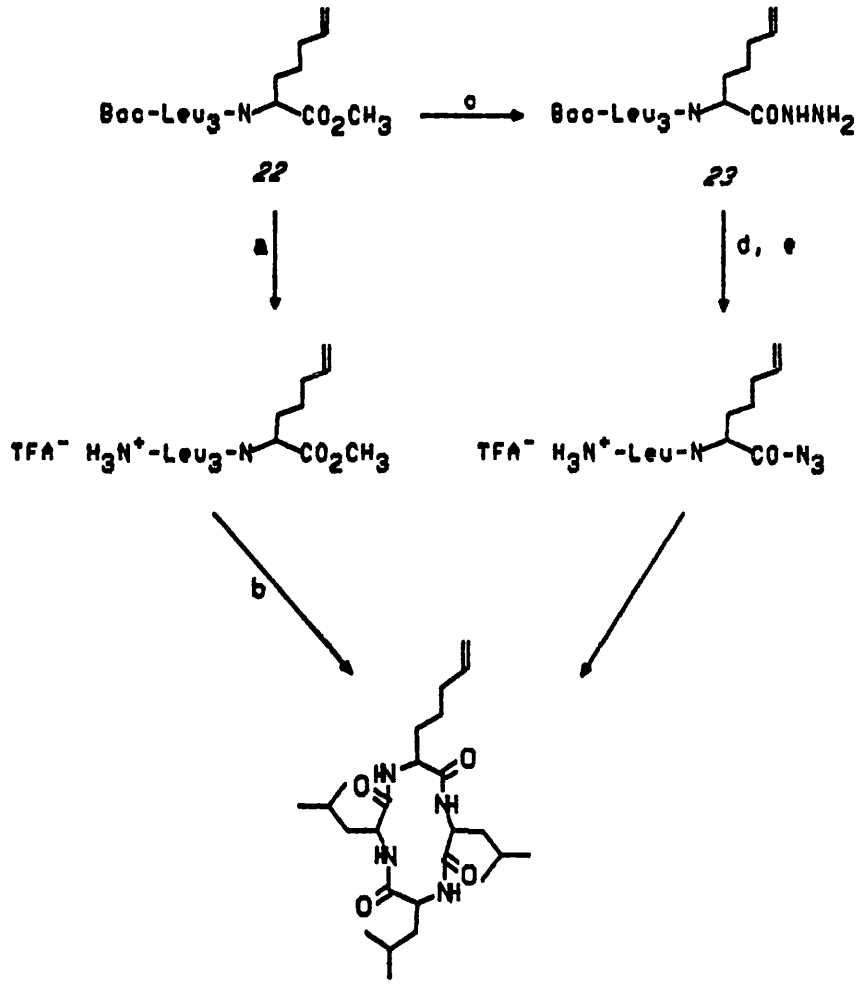
were found which were more conducive to cyclisation than to cyclo-dimerisation.[83] The coupling method applied for ring

closure also plays some role, for example, cyclo-dimerisation is less pronounced when the open chain precursor is activated in the form of its azide[84] than in cyclisation by other procedures. A more decisive influence is exerted, however, by the concentration of the peptide subjected to cyclisation. The principle of dilution[85] must be adopted in cyclisation, if a uni-molecular reaction is the aim. At high concentrations of the activated peptide, bimolecular dimerisation, and polymerisation reactions emerge as serious competitors.

8.11.1 Attempted cyclisation methods.

Method a:- The linear tetra-peptide (22) was de-protected in the same manner as for the di-peptide (11) using TFA/anisole (Scheme 8.16). Using the method of Schywzer and Seiber,[86] an attempt at cyclisation was made to afford the cyclic tetra-peptide (24). However, cyclisation by this method proved unsuccessful primarily because the peptide was protected as its methyl ester, which is relatively un-reactive towards nucleophilic attack by the terminal amino function. The starting material, the de-protected linear tetra-peptide methyl ester, was recovered.

Method b:- The azide method of cyclisation, based on the method of Bodansky and Hemes,[87] was also attempted. Azides are frequently used for cyclisation of peptides of different chain lengths. The starting materials are the usual protected hydrazides. The amino protecting group is



a:- TFA/anisole, 1h, RT; b:- DMF/pyridine, 65°C, 72h; c:- MeOH, $NH_2NH_2.H_2O$, 48h, RT; d:- TFA/anisole, 1h, RT; e:- azide formation via butylnitrite.

Scheme 8.16

removed, the hydrazide converted to the azide, and the activated peptide allowed to cyclise. This is possible because the reaction of nitrous acid with the hydrazide group is considerably faster than that with the N-terminal amino group.[88] Thus the amino group remains essentially intact during the conversion of the hydrazide

to the azide. Also, because the reaction is carried in an acidic medium, the amino group is protonated, and becomes inert towards attack by the azide group, it is possible and practical[89] to dilute the solution of the azide, and to expose afterwards the amino group to acylation afterwards (Fig 8.51).

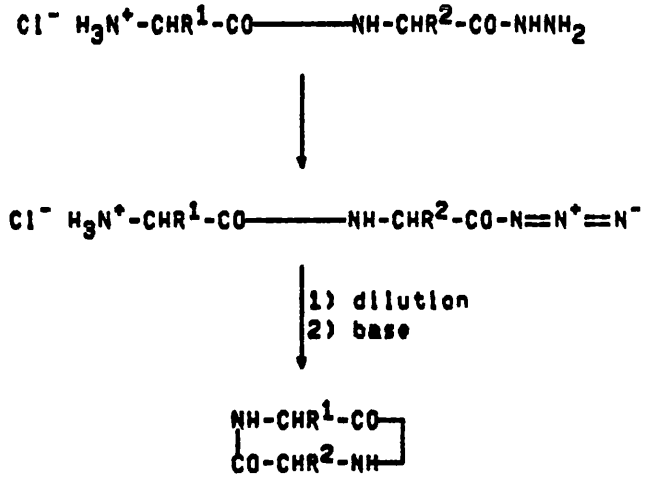


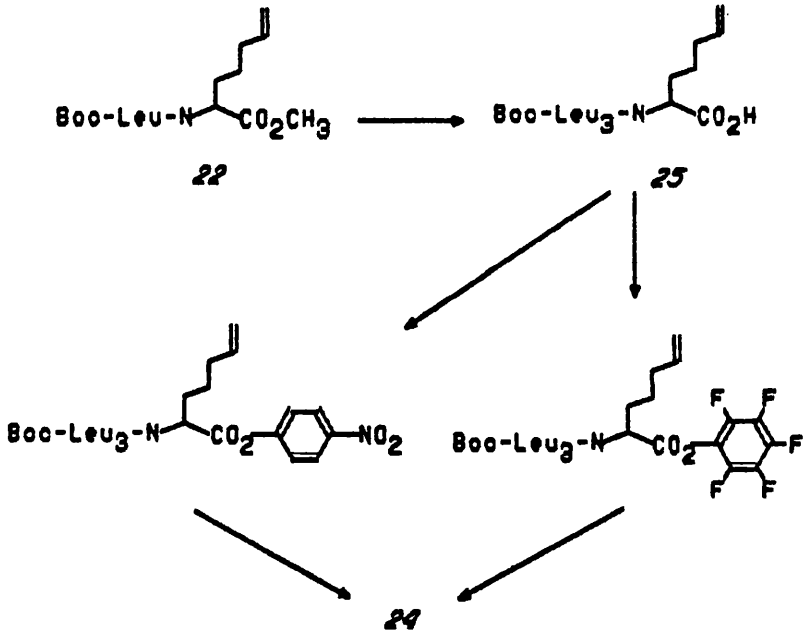
Fig 8.51

However, when this method was applied to the hydrazide (23), a multi-component mixture was formed, which was not chromatographed or characterised. Several further attempts using this method proved equally unsuccessful, resulting in a multi-component reaction mixture in each experiment.

8.11.2 Other methods of cyclisation.

Because of the lack success of the two methods described above, a third cyclisation route was explored. This involves hydrolysis of the linear tetra-peptide methyl ester (22) to the free carboxylic acid, and subsequent re-

esterification to a more activating ester group, such as p-nitrophenyl or pentafluorophenyl esters (Scheme 8.17).



Scheme 8.17

Hydrolysis, using aqueous NaOH, of the linear tetra-peptide (22) has already been demonstrated, following the method originated by Iselin *et al*,[31] to afford the free carboxylic acid peptide (25) in 96% yield. This is in contrast to the previous attempts at hydrolysis on the methyl ester derivatives of compounds (10) and (20) (46% and 16% yield respectively) using the same method. Analysis of ^{1}H and ^{13}C NMR data showed that the linear peptide was intact, and that the OCH_3 group is no longer present.

The choice of the new ester group must be such that the reactive intermediates must be stable enough to withstand the unmasking of the N-terminal amino group. Thus formation of either the p-nitrophenyl or pentafluorophenyl

ester seem suitable, since both activate the carbonyl carbon sufficiently to nucleophilic attack from the amino end, and both are good leaving groups, by virtue of the stability of the anions they form. The use of p-nitrophenyl esters has already been described by Schwyzer and Sieber[89] in the synthesis of gramicidin S.

Synthesis of the pentafluorophenyl ester can be achieved using the method of Kisfaludy and co-workers,[89-91] and subsequent cyclisation of the ester by the method of Sheh and Mokotoff.[92]

Alternatively, activation of the terminal carbonyl group of a peptide with an unprotected amino group can be used, but this is perhaps the more problematic alternative, since the method of activation must be selected with care. For instance, the reaction of free peptides with alkylchloroformates, should produce not only the mixed anhydride, but also urethanes, in which cyclisation is blocked (Fig 8.52). As mixed anhydrides are potent acylating agents, protonation of the amine does not provide complete protection. Yet in spite of this complication, cyclisation attempts via mixed anhydrides has provided valuable results,[93,94] if ring formation is favoured by the geometry of the molecule. More favourable consideration can be given to coupling reagents, since these can activate carbonyl groups in the presence of amines.

Carbodimides can be particularly useful, because the rate of their reaction with amines is negligible, in comparison with the rate of acylation of carboxylic acids.

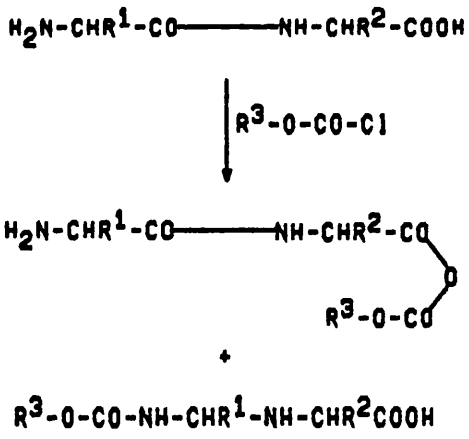


Fig 8.52

On the other hand, while the reactive intermediates are potent acylating agents, if the cyclisation is impeded by an unfavourable geometry, they will produce, through O->N acyl-migrations, *N*-acylurea derivatives, which contaminate the expected cyclic peptide (Fig 8.53). It is therefore not

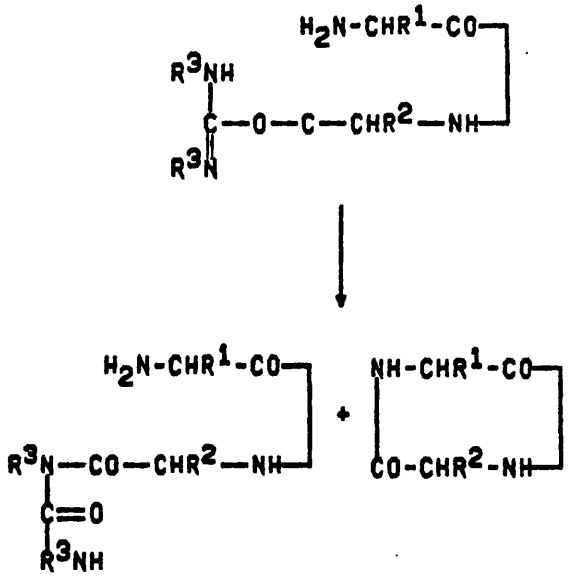


Fig 8.53

too surprising that low yields are often observed in cyclisations with carbodimides, even when the reagent is applied in high concentration.[96] Better results can be achieved if the conformation of the open pre-cursors favours cyclisations, as in the case of some hexa-peptides.[97]

Improvement might be expected if the O->N acyl-migration is suppressed by the addition of auxiliary nucleophiles, such as *N*-hydroxysuccinimide[98] or HOBt. Cyclisation with other coupling reagents, such as *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodimide, may suffer similarly from the disadvantage of O->N acyl-migration, forming inactive *N*-acylurea. Yet in the synthesis of more simple cyclo-peptides fairly good results were obtained[99] when the azides were generated from peptide hydrochlorides with the help of DPPA.[100] Another consideration is that, although coupling reagents are convenient to use, the activation and cyclisation steps cannot be separated. The activation step requires a high concentration of the coupling reagent and peptide, while cyclisation competes favourably with polymerisation at low concentration. In general, an excess of the coupling reagent is used, with the peptide at low concentration.

At present it would appear advisable to make a clear separation of activation and coupling. For example, in the attempted cyclisation of a hepta-peptide, with the sequence of evolidin, no tangible product was formed when the reaction was carried with the aid of DCCI, but a cyclo-

hepta-peptide, with the properties of natural evolidin, could be secured through the conversion of the *N*-protected open chain intermediate to the *p*-nitrophenyl ester, followed by de-protection of the *N*-terminal amino group which led to ring closure.[101]

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CHAPTER NINE.

SILICON CHEMISTRY.

9.1 Introduction.

The next objective of this project was to chemically bond the cyclic peptides, *13*, *14*, *18*, and *19*, described in the previous chapter, onto the silicon centres of mononuclear silanes, and onto polysiloxanes. These could then be used to bond or coat the peptides, via an alkyl spacer chain, onto a variety of surfaces containing hydroxyl groups, such as silica or alumina. Alternatively the functionalised siloxanes could be used in the form of neat peptide functionalised fluids as supported liquid membranes, or in liquid-liquid partition chromatography. Initially, attention was directed towards bonding such compounds onto HPLC silica gel (Chromspher Si, 5µm, spherical), and subsequently testing the capability of such stationary phases for enantiomer separations.

The development of column packings with covalent bonded phases is one of the factors that has led to the growth of HPLC during the past decade or so. To form bonded phase packings, silica typically is reacted with functionalised silanes (Eq 9.1), which contain one or more reactive groups (X), such as alkoxy or halide, and R is typically an alkyl or substituted alkyl group. This creates siloxane bonds between the silane and the silicon atoms of

the solid support, giving a stationary phase where the R groups of the silane largely determine the chromatographic behaviour of the column packing.

Eq 9.1 $\quad Si\text{-}OH + SiX_nR_{4-n} \text{ ---> } Si(OSiX_{n-1}R_{4-n})$

There are several variations in the type of reaction possible. Reaction with a monofuctional silane (n=1) may yield a surface monolayer. The reaction is reproducibile (Fig 9.1) and convenient, with the resultant phase exhibiting good mass-transfer properties, and producing high column efficiencies.

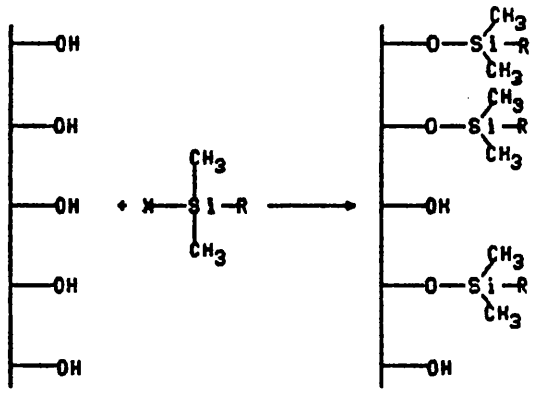


Fig 9.1. Representation of monofunctional silanes bonded to a hydroxylated surface.

Bifunctional or trifunctional silanes may react with only one or two surface silanols[1] so leaving Si-X residues which on exposure to moist air will hydrolyse to form new silanols groups (Fig 9.2). Such behaviour can be can especially detrimental in subsequent usage for the

separation of basic solutes. A silanisation reaction using such reagents in excess can also produce a partially

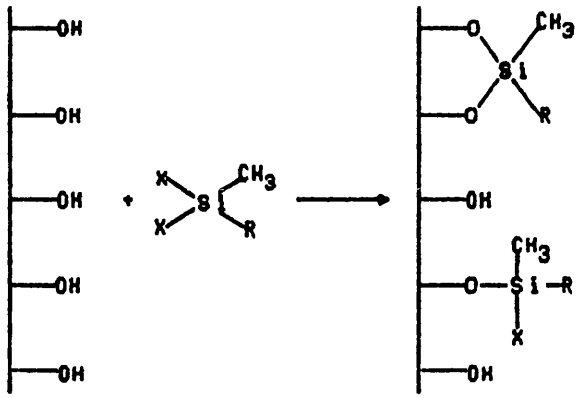


Fig 9.2. Representation of polyfunctional silanes bonded to a hydroxylated surface.

polymerised layer on the surface of the support following hydrolysis and curing (Fig 9.3). Polymeric phases have some

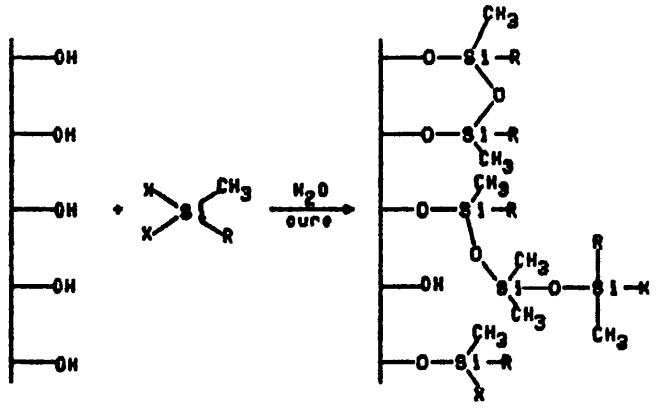


Fig 9.3. Representation of polyfunctional silanes bonded to a hydroxylated surface.

advantages over monolayer phases, in that the former are more resistant to hydrolysis, and partially mask residual acidic silanol surface groups. However, this type of phase is more difficult to form reproducibly, and variations in total organic content are often observed from one preparation to the next. Mass-transfer characteristics of such phases can be very poor[2] if the stationary phase is relatively thick, leading to poor column efficiencies.

The stability of bonded phase packings in HPLC is always an important consideration. Providing these materials are used in the pH range 4-7, in which the Si-O-Si linkage is stable, the organic proportion of the mobile phase is large (>20%), and a column temperature of $<60^{0}C$ is maintained, the separation of many basic compounds, such as pharmaceutical and agricultural chemicals, also unprotected peptides and proteins, can be effected using reverse-phase HPLC. Typically, aqueous/organic mobile phases containing TFA are used[3] in peptide and protein separations because:- a) samples are soluble in TFA containing solvents; b) bio-molecules then produce good peak shape; and c) TFA can be easily removed by lyophilisation prior to further characterisation of the separated species. However, Kirkland and co-workers[4], have shown that these columns are not stable, at low pH (<3), even over relatively short periods of time. They demonstrated that commercial HPLC columns with dimethylalkyl silane bonded phases showed a significant loss of bonded phase during use with low pH and TFA/water

acetonitrile gradient elution separations. In addition some bonded phases also change in nature, and both of these effects are highly detrimental in chromatographic separations.

Kirkland and co-workers[4] then developed two new types of bonded phase that showed significantly increased stability over conventional types of columns. One uses bifunctional (or didentate-type) silanes, which contain one reactive site on each of two silicon atoms of the silane (Fig 9.4). The two silicon atoms are connected by variable bridging groups, such as -O- or $-(CH_2)_n$ etc., in order to adjust the spacing between the silicon atoms of the silane

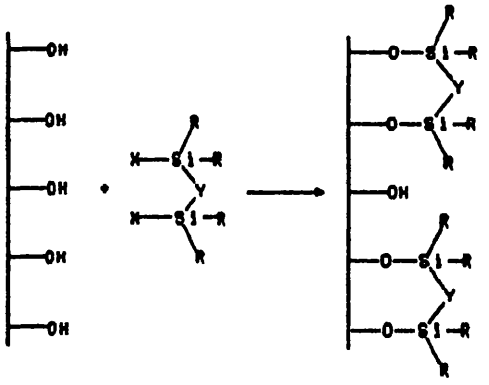


Fig 9.4. Representation of bidentate silanes to a hydroxylated surface.

for the most favourable reaction with the SiOH sites on the surface of the silica support. Bonded phase packings with methyl, vinyl, phenyl, isopropyl, and tert-butyl functional groups exhibited similar properties, but with greater stabilities at low pH, than their corresponding

dimethylsilyl derivatives.

The second type of novel bonded material employs monofunctional silanes which contain one or two bulky substituents, such as isopropyl or *tert*-butyl. The bulky groups provide steric protection against hydrolysis at low pH, to the Si-O-Si bond on the surface of the silica support while still providing equivalent retention and column efficiency for analysis (Fig 9.5).

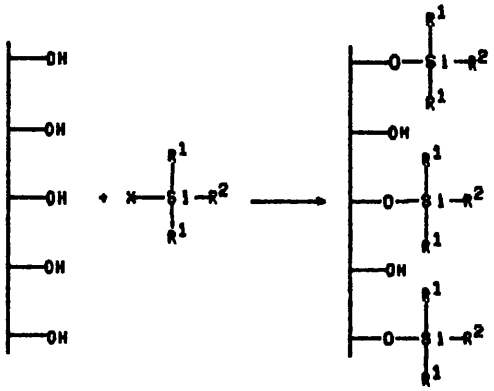


Fig 9.5. Representation of sterically protected monofunctional silanes bonded to a hydroxylated surface.

RESULTS AND DISCUSSION.

9.2 Synthesis of silane and polysiloxane attached peptides.

Three model reactions were first performed using allyl benzene, and dichloromethylsilane under differing reaction conditions in order to gain experience in the method before

the target peptides were employed. Two reactions between allyl benzene and triethylsilane were also carried out, in order to determine whether steric factors would prevent complete reaction, as this could also be significant for bulky peptides containing short spacer chains. Reaction with short-chain commercially-available polysiloxanes containing both Si-H and $SiMe_2$ units (3,5-siloxane polymer)

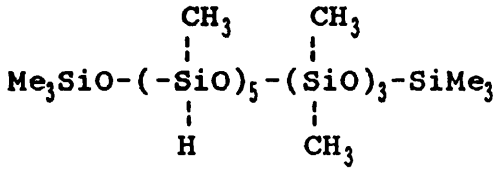
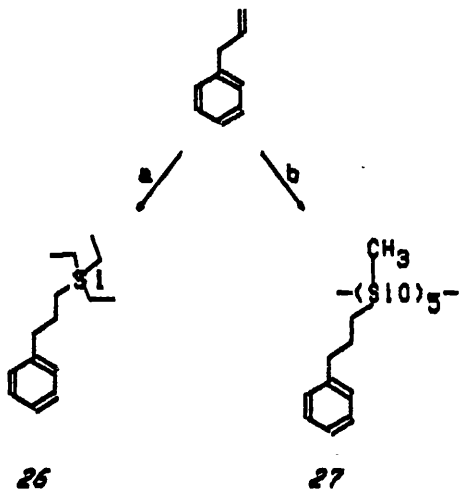


Fig 9.6

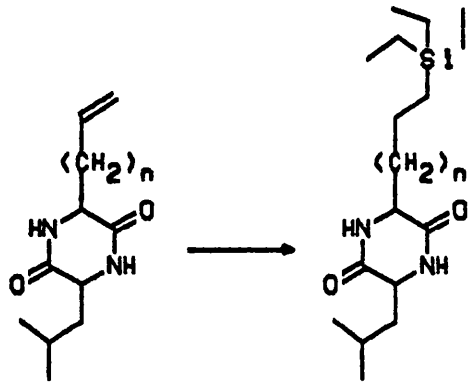
was also performed (Fig 9.6) using chloroplatinic acid ($H_2PtCl_6.xH_2O$, Spiers catalyst) for the hydrosilylation reaction. This proved satisfactory for the allylbenzene-Et_3SiH reaction, although yields were lower than expected (Scheme 9.1). This may have been due to steric effects or the presence of water. The reaction between allyl benzene and the 3,5-siloxane polymer afforded the required product (Scheme 9.1) in 88% yield.

Table 9.1 lists the reaction conditions, solvent combinations, and the catalyst used for the reaction of the peptides with various silicon moieties. Reaction of the cyclic peptides with Et_3SiH was successful (Scheme 9.2), with yields ranging from 38-87%. The conditions for these reactions were quite different from analogous reactions involving allylbenzene, and will be discussed later.



26 27

Scheme 9.1. a:- Et_3SiH, Toluene/butan-2-ol, H_2PtCl_6 catalyst, reflux, 24h; b:- 3,5-siloxane polymer, H_2PtCl_6 catalyst, reflux, 48h.



Scheme 9.2. Toluene/MeCN, Cp_2PtCl_2, EtSiH, heated in sealed reaction tube.

Attempts to synthesise a siloxane attached cyclic peptide by catalysed reaction of the cyclic peptide (*13*, *14*, *18*, or *19*) with the 3,5-siloxane polymer, yielded a multi-component mixture of siloxanes containing various peptide loadings. Attempts to chromatograph these mixtures on silica gel proved unsuccessful, resulting in the appearance, on elution, of several new components. It seems

probable that the steric size of the cyclic peptide prevents complete reaction, thus leaving a mixture still containing Si-H bonds. As silica surfaces are particularly acidic, they will bond the short chain siloxane to the surface via reaction of the remaining Si-H groups. Attempts

Table 9.1. Conditions used for the reaction between the cyclic peptides and various silicon moities (Scheme 9.2).

Si moiety	n	Solvent/s (v/v)[a]	Catalyst	Reaction conditions[d]
Me -(SiO)2-[b] H	1	Tol/Bu-2-OH (2:1)	H_2PtCl_6	reflux, 24h
	1	Tol/Bu-2-OH (2:1)	H_2PtCl_6	reflux, 96h
	1	Tol/MeCN (2:1)	H_2PtCl_6	80-90°C, 6 days
	1	Tol/BuOH (2:1)	H_2PtCl_6	80-90°C, 3 days
	1	Tol/MeCN (2:1w	H_2PtCl_6	80-90°C, 6 days, N_2
	3	Tol/MeCN (2:1)	H_2PtCl_6	ultra-sound bath, 24h, sealed system
	3	DCM	H_2PtCl_6	ultra-sound bath, 24h, sealed system
	3	Toluene	H_2PtCl_6	reflux, 3 days, N_2
	3	Toluene	H_2PtCl_6	80-90°C, 3 days
	3	Toluene	H_2PtCl_6	90-100°C, 48h, N_2

Et_2SiH_2	3	Tol/MeCN (2:1)	H_2PtCl_6	50-60°C, 3 days
	3	MeCN	Cp_2PtCl_2	90°C, 5 days, sealed tube
$-[MeSi(H)O]_2$[c]	1	MeCN/Tol (70:2)	H_2PtCl_6	80°C, 24h
$MeSiH(OMe)_2$	1	DCM	H_2PtCl_6	reflux, 24h, N_2
Et_3SiH	1	t-BuOH	H_2PtCl_6	100°C, 24h
	1	Tol/MeCN (1:3)D	H_2PtCl_6	150°C, 48h, N_2, sealed tube, (87%)
	3	Tol/MeCN (1:3)D	Cp_2PtCl_2	80°C, 32 days, N_2, (45%)
	3	Tol/MeCN (1:3)D	Cp_2PtCl_2	150°C, 8 days, N_2, sealed tube, (48%)
	3	Tol/MeCN (1:3)D	Cp_2PtCl_2	150°C, 48h, N_2, sealed tube, (31%)

a:- numbers in parenthesis refer to the ratio of solvents by volume. D, degassed solvents; b:- $Me_3Si-(OSiMe_2)_3-(OsiHMe)_5-OSiMe_3$; c:- $[(Me_3SiO)(SiMeH_2)_2]O$; d:- numbers in parenthesis refer to reaction yield.

to elute these materials led to fragmentation. Neither alumina nor fluorosil, proved suitable for chromatographic

separation of the products. Gel permeation chromatography was also used in an attempt to identify the product/s, but with inconclusive results. Only in one reaction was the required reaction achieved, and the NMR spectrum of the completely reacted major product could be completely assigned. However, this result could not be reproduced, and the reaction was finally abandoned.

Reaction of the cyclic peptide with other siloxanes (Fig 9.7) was also attempted, but again either no reaction was evident, and the starting material was recovered, or a multi-component mixture was produced from which it was not possible to isolate or identify positvely the individual components.

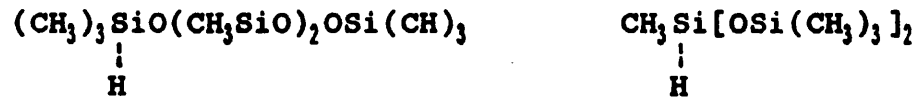
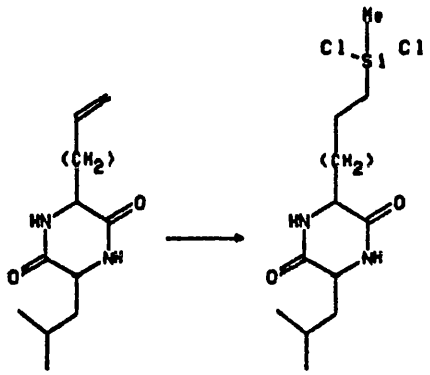


Fig 9.7

In the second model study, the synthesis of dichlorosilane derivatives (32) were attempted, using a similar method to that described by Pirkle and Pochapsky[5] (Scheme 9.3). The product was expected to be extremely easily hydrolysed on contact with moist air, and reactions were therefore performed with careful exclusion of moisture. Due to the difficulty of handling chlorosilanes only ^{1}H NMR data is available on the product, but it shows that the cyclic peptide remained intact throughout the

course of the reaction, despite the very large excess of chlorosilane used in the model study.



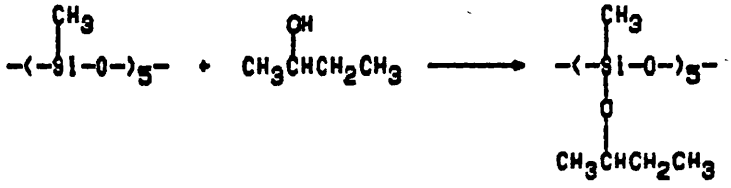
Scheme 9.3. Toluene/DCM, H_2PtCl_6, $MeSi(Cl)_2H$, R.T., 48h, reflux 30min or reflux 48h.

Transference of the dichloromethylsilyl derivative of the peptide (see below) into a stirred suspension of silica gel in toluene, was done via a cannular, under nitrogen gas. In the initial study of the peptide modification of silica gel (12µm, 90% ±7µm), triethylamine was added to bind any HCl liberated. Subsequently this was omitted in order to avoid any possibility of the base de-activating the silica gel.

9.2.1 Effect of solvent composition on hydrosilylation reactions of peptides.

The choice of solvent was found to be a very important factor in determining the success of the reaction. The solvent was required to dissolve the cyclic peptide, but not compete in the reaction, nor boil at too low a temperature. In many hydrosilylation reactions, involving

liquid reactants, no solvent is used.[6-9] In this reaction a mixture of toluene and butan-2-ol was used initially, as the peptides were prepared in this solvent combination, and are soluble in it. However, it appeared from NMR evidence on the reaction products, that butan-2-ol reacted with the Si-H groups in the siloxane polymer. This was confirmed by heating the solvent combination with the 3,5-siloxane polymer in the presence of catalyst, to afford the appropriate product (*28*) (Scheme 9.4), which was identified by NMR spectroscopy. Although primary alcohol ≡Si-H reactions are facile, sterically hindered secondary alcohols are generally slow to react in the presence of a 1-alkene, and a platinum catalyst. This suggests that hydrosilylation of the alkenyl functionalised peptide might require forcing conditions.



28

Scheme 9.4

Other solvent systems evaluated included toluene alone or mixed in various proportions with *tert*-butanol, or acetonitrile, dichloromethane, and also pure *tert*-butanol. Toluene/acetonitrile was finally chosen as this allowed a fairly high temperature to be used during reaction, and was easier to handle than *tert*-butanol, which although

chemically suitable, is a low melting point solid.

9.2.2 Mechanism of hydrosilylation.

In general, hydrosilylation involves the addition of an Si-H unit to an unsaturated molecule (Fig 9.8). The reactions are usually accomplished by one of the following methods:-

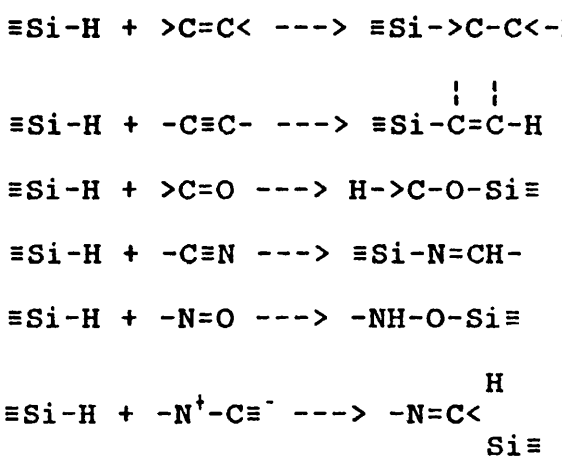


Fig 9.8

a) by heating the reactants together, usually at 300^{0}C or higher under high pressure

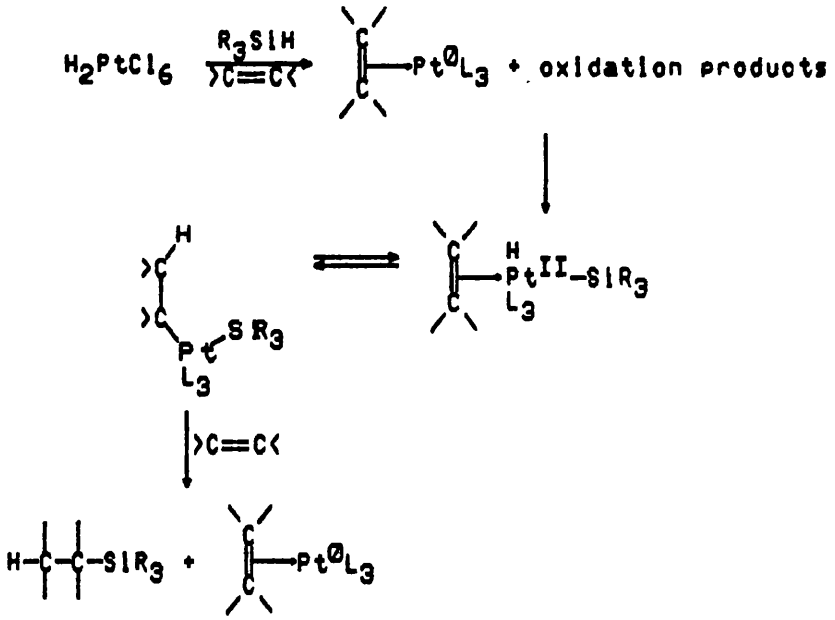
b) by using a radical initiator, such as an organic peroxide or an azo compound

c) by exposure to UV- or γ-radiation, or electrical discharge

d) by catalysing the reaction with transition metal salts, such as H_2PtCl_6, bis(dicyclopentadienyl)-platinum dichloride, Cp_2PtCl_2, $Co_2(CO)_8$ etc., or a Lewis base, such as pyridine.

Platinum salts are the catalyst of choice for 1-alkene

reactions, and either H_2PtCl_6 or Cp_2PtCl_2 was used in this study. The catalyst may be used homogeneously or heterogeneously and in some supported catalyst systems, reaction is thought to occur at metal sites which remain bonded to the support during the reaction.[10] Both the type and identity of the catalyst affect the overall yields and selectivities. The suggested mechanism for platinum catalysed addition[8] is shown in Scheme 9.5.[NB]



Scheme 9.5

In general, the presence of electron-withdrawing substituents on the alkene will reduce reactivity, this effect being related to the ease of initial complex formation. The addition is tolerant of a wide variety of functional groups on both carbon and silicon, but a strong

---

NB It is assumed that a similar mechanism exists for Cp_2PtCl_2

steric influence is exerted, with silicon bonding to the less hindered end of the alkene. It has been shown that the structure of the ≡Si-H containing compound can play an important role in determining the position of the Si--C bond in the alkyl groups formed by hydrosilylation.[11] For example, the addition of $Cl_xMe_{3-x}SiH$ (x=3, 2, 1) to phenylalkenes in the presence of H_2PtCl_6 yields two products (Fig 9.9).[7] Terminal alkenes are hydrosilylated more readily

$Ph(CH_2)_{n+2}SiCl_xMe_{3-x}$ and $PhCH(SiCl_xMe_{3-x})(CH_2)_nCH_3$

Fig 9.9

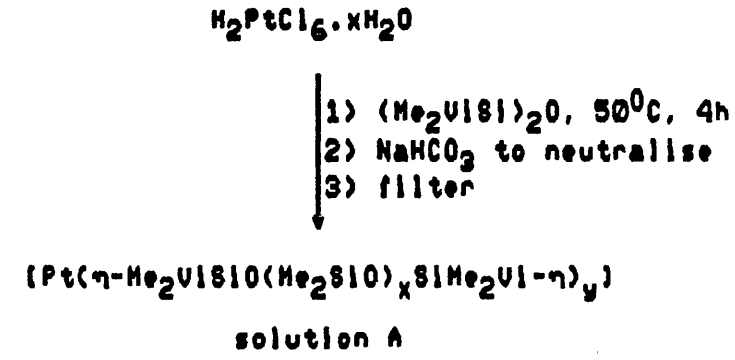
than internal alkenes, which are known to isomerise prior to addition, so yielding terminal products in many instances (Fig 9.10).[7]

3-heptene $\xrightarrow{HSiR_3}$ 1-heptene $\xrightarrow{HSiR_3}$ n-$C_7H_{15}SiR_3$

Fig 9.10

In an attempt to define more precisely the platinum catalytic species, Chandra et al[12] studied the reaction of Spiers catalyst with *sym*-tetramethyldivinyldisiloxane $(Me_2ViSi)_2O$ (Vi=vinyl), (solution A) (Scheme 9.6). They suggest that this reaction generates Pt(0) species, their evidence coming from:- a) the isolation and characterisation of a three-co-ordinate complex, $[Pt\{(\eta CH_2CHMe_2Si)_2O\}(P\text{-}t\text{-}Bu_3)]$, after addition of P-t-$Bu_3$ to

solution A; b) the high yield synthesis of other Pt(0) complexes from solution A (Table 9.2); and c) cyclic



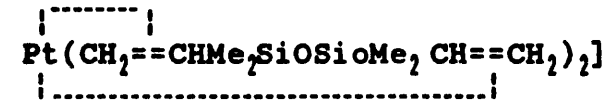
Scheme 9.6

Table 9.2. Pt(0) complexes synthesised by Chandra et al[12] from reaction with solution A.

Reactant	Product	Yield (%)
PPh_3	$[Pt(PPh_3)_3]$	85
diphos	$[Pt(diphos)_2]$	76
PR_3	$[Pt\{(\eta CH_2CHMe_2Si)_2O\}(P\text{-}R_3]$	
	R=t-Bu	79
	R=Cy[a]	63
$2RC{\equiv}CR'$[b]	$[Pt(\eta\text{-}RC{\equiv}CR')_2]$	
	R=Ph, R'=CMePhOH	85
	R=R'=Ph	56
	R=R'=CMe_2OH	64

a:- Cy=C_6H_{11}

voltammetry, ^{195}Pt NMR data, and gas chromatographic evidence. Chandra et al[12] suggest that the initiator in the $H_2PtCl_6.xH_2O$-vinylsiloxane hydrosilylation system is a bis(η-vinyl)-chelating Pt(0) complex:-



being the principal ingredient. Furthermore, a convenient source of Pt(0) complex can be obtained by treating $H_2PtCl_6.xH_2O$ with a vinylsiloxane, which is a simpler alternative to "naked" platinum complexes, such as [Pt(η-C_2H_4)$_3$] or [Pt(COD)$_2$] (COD=1,5-cyclo-octadiene).

9.2.3 Other factors affecting hydrosilylation.

Successful hydrosilylation reactions were carried out in a sealed reaction tube in order to permit high pressure conditions. The design of the tube (Fig 9.11) was such that all the solvents and reactants had minimal contact with air and a positive pressure of nitrogen gas was maintained above the reaction mixture.

All solvents used were degassed by bubbling nitrogen gas through them for ca 30min. This was found to maintain the activity of the catalyst, which otherwise is reduced to the metal, as seen by a darkening of the reaction mixture before the reaction is complete, and after the "induction period",[11] which is often observed before any reaction

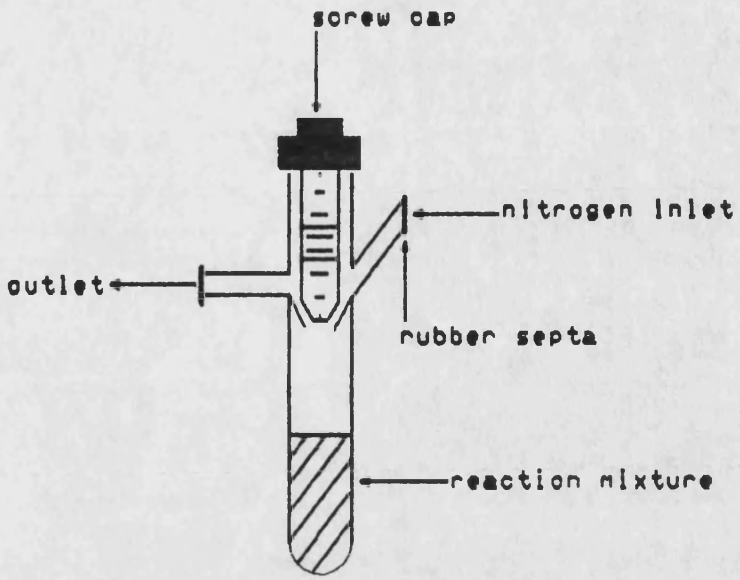


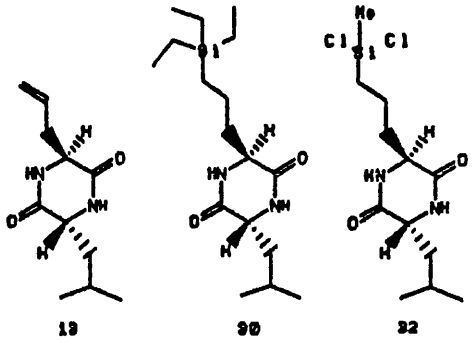
Fig 9.11. Design of sealing reaction tube used in the reactions between the cyclic peptides and triethylsilane. Tube capacity was ca 6cm^3 and 12cm^3.

proceeds.

9.2.3 Analysis of results.

In all cases a 2-5 fold excess of the silane or siloxane was used in the reaction. All reactions involving Et_3SiH were successfully chromatographed on silica gel. NMR data also confirmed that no unreacted 1-alkene remained. Thus signals due to $-CH=CH_2$ in the region 5-6ppm were absent in ^1H NMR spectra were replaced by signals at ca 1.81 and 0.91ppm for *(30)* and ca 1.33 and 0.50ppm for *(31)* (Table 9.3 and 9.4). Of particular note are the changes in chemical shift of the NH protons of *(13)*, *(30)* and *(18)*, *(31)* due to the shielding effect of the silicon atom, on addition of the Et_3Si-group to the double bond. A de-shielding effect is experienced by the NH protons of *(32)*

Table 9.3. Effect of silicon moiety on the chemical shift (δ_H ppm) of selected protons of the cyclic peptide.



13 30 32

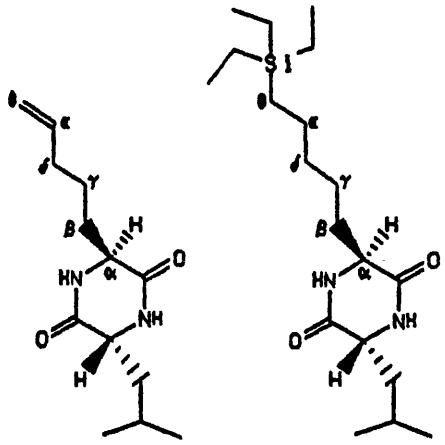
	NH Gly	NH Leu	αCH Gly	αCH Leu	βCH$_2$	γCH$_2$	δCH$_2$
13[a]	6.21	6.48	4.04	4.00	2.83	5.24	5.76
30	7.22	7.40	3.95	3.92	ca 1.90	1.81	0.91
32[b]	7.51	7.64	4.27	4.20			

a:- Protons γ and δ for *13* refer to the terminal double bond; b:- NMR solvent CD_2Cl_2, all others in CD_3Cl.

on addition of the Cl_2MeSi-group.

Addition of the silicon moiety occurred at the terminal carbon atom, as opposed to the penultimate carbon atom (**Fig 9.12**). However, due to the short length of the spacer chains, it seem likely that steric hindrance, would strongly favour addition at the terminal carbon atom. The yields of the triethylsilyl derivatives ranged from 87% for (30) to 38% for (31). The lower yield of (31) is due mainly to incomplete reaction.

Table 9.4. Effect of silicon moiety on the chemical shift (δ_H ppm) of selected protons of the cyclic peptide.



18 31

	NH Gly	NH Leu	αCH Gly	αCH Leu	βCH_2	γCH_2	δCH_2	εCH_2	θCH_2
18	8.15	8.04	3.97	3.96	1.82	1.62	2.10	5.78	5.05
31	7.27	7.22	3.98	3.96	ca 1.33	ca 1.33	ca 1.33	ca 1.33	0.50

9.3 Attachment of the cyclic peptide to silica surface.

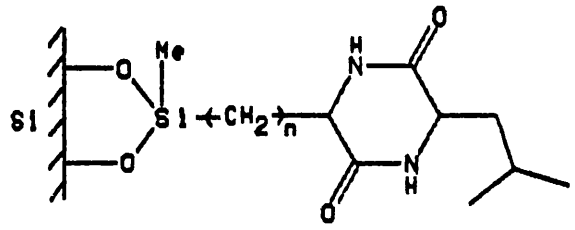
The attachment of the cyclic peptide, via a spacer chain, to the surface of the silica gel was achieved via a reactive dichloromethyl silane intermediate, Both the extent and permanency of the attachment, as well as the spacer chain length, are of importance for applications of this material.

9.3.1 Extent of modification.

Modification of the silica gel has already been discussed above. As discussed earlier, the properties of a silica bonded phase are profoundly affected by coverage. For adsorbents, the bonding reaction is determined by the type, the activity, and steric factors affecting the reactive sites at the silica surface.[13] Colin and Guiochon[14] suggest that the coating density increases with increasing temperature at first, and becomes constant above 75°C.

Qualitative carbon analyses can be used to measure coverage density of the silca gel. While giving an indication of the extent of the reaction between silanols and chlorosilane molecules, such analyses is considered to be misleading.[1,15] The extent of the modification/loading, in this study, is expressed in mMg^{-1} of silica gel (Table 9.5), and the figures corrected for, by comparison with an untreated sample of silica. Loadings were calculated by comparison of the theorectical and observed elemental analyses. Preliminary modification experiments using HPLC grade silica gel, yielded a stationary phase with loadings lower (ca 3-5 times lower) than those tabulated in Table 9.5. Loadings were increased by oven drying the silica gel prior to modification with the cyclo-peptide. The loadings calculated for *CSP 2* and *3* are comparable to those obtained by Pirkle and co-workers,[5,16] who obtained values ranging from 0.23-0.37mMg^{-1} and 0.19-0.30mMg^{-1}, based on carbon and nitrogen analyses respectively.

Table 9.5. Corrected elemental analyses of modified silica gel and calculated loadings.



n=3,5

CSP	n	Elemental analysis (%)			Loading (mMg^{-1} of silica gel)		
		C	H	N	C	H	N
2	3	4.93	0.66	0.95	0.34	0.30	0.34
3	5	4.82	0.62	0.75	0.29	0.24	0.27

9.2.2 Oven curing.

The modified silica was oven cured in order to increase its stability, as there is evidence that relatively few covalent bonds are formed on completion of the grafting reaction.[17] The effect of oven curing was studied by Waddell et al[17], who studied silica immobilised (3-aminopropyl)triethoxy (81), methyldiethoxy (82), and dimethylethoxy (83) as models. They found that oven curing times of 3h at 80°C were sufficient to achieve maximum silane-surface stability. At zero curing times, they found the order of stability of each type of silica to be (81) >

(82) > (83), and concluded that the number of covalent bonds initially formed to the surface was related to the number of silanol groups, and primarily determines the stability after isolation.

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CHAPTER TEN.

LIQUID CHROMATOGRAPHY.

10.1 Introduction.

10.1.1 Enantiomeric separations on chiral stationary phases.[1]

For enantiomeric separation to occur, two conditions must be fulfilled. Diastereomeric complexes must be formed between the CSP and at least one of the enantiomers, and these must also differ in their free energy of formation. The latter has already been discussed (CHAPTER 4 section 4.5.)

The formation of diastereomeric complexes occurs as a result of attractive interactions between the analyte enantiomers and the CSP, removal of the analyte enantiomers from a reverse mobile phase, or by passive diffusion of the analyte enantiomers into a chiral environment. In the latter case, chiral recognition is sterically controlled, and can be likened to a stationary phase containing chiral cavities, in which chiral recognition results from the ability of the individual enantiomers to "enter" the chiral cavity.

Free energies are often small when chromatographic separation of enantiomers involves interaction between CSP and analyte, implying that the molecules are free to

"tumble" and exert little mutual conformational control with respect to each other. The diastereomeric complex having the lowest energy conformation will principally determine the degree of enantioselectivity observed. An important note to recognise is that it is the time weighted average of all possible interactions that is important for determining the retention and enantioselectivity. In some cases, more than one chiral recognition process may be involved in contributing to the overall chromatographic behaviour.

The efficiency of the system employed also influences the difference in free energy, δG, and hence the chromatographic separations observed. If the system employed is highly efficient, so that narrow peaks are observed, δG need only be relatively small, eg., small-scale analytical separations. For such applications peak separation is the main requirement, and enantioselectivity is of secondary importance. High enantioselectivity is, however, required for preparative separation of enantiomers, this requiring an increase in δG, thereby increasing enantioselectivity of the CSP.

It should be noted that no attempt to proposed a mechanism of separation is made, and the reader is referred to several recent articles on the mechanistic aspects of enantiomeric separations using chiral stationary phases.[2-4]

RESULTS AND DISCUSSION.

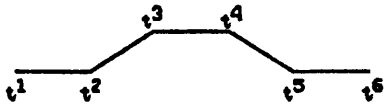
10.2 Liquid chromatography.

A model column (*CSP 1*) was slurry packed, using 4.0g of the modified stationary phase and propanol ($30cm^3$), into a stainless steel column (20 x 0.49 I.D. cm) under high pressure. The column was conditioned using methanol, hexane, and 50% methanol/water. The test columns (*CSP 2* and 3), with dimensions 25 x 0.45 I.D. cm, were similarly treated. A guard column (50 x 0.49 I.D. cm) was filled with silica. All columns were fitted with Swagelok female fittings, and 2µm stainless steel frits.

The following equipment was used:- Gilson piston pumps, model 303; Gilson dynamic mixer, model 811B; Gilson manometric module, model 802 or 802C; Rheodyne syringe loading sample injector, model 7010 fitted with a 20µl loop or model 7125 fitted with a 50µl loop; LDC/Milton Ray UV Spectromonitor III or Gilson UV Detector, model 116, both with UV detection at 214nm; Gilson HPLC System controller, model 702 V3.0 or 714 V1.2 with Gilson systems interface module, model 506B.

All solvents were of HPLC grade. Water was distilled, and TFA was distilled from $KMnO_4$. Table 10.1 lists the solvents used and the gradient elution profiles. System I is frequently used in the laboratory for the qualitative analysis of peptides and amino acids using a C_{18} HPLC column. System III has been frequently used with chiral stationary phases,[5-10] and was used to study the effect of a

Table 10.1. Gradient elution profiles of System I, II and III used for testing ***CSP 1***, **2** and **3**.



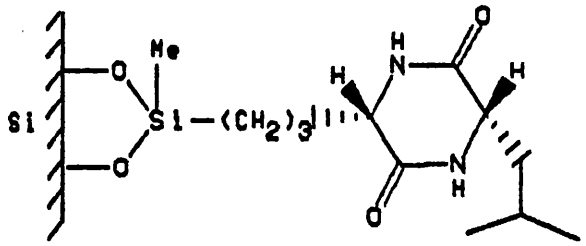
	Gradient elution profile (t/min, % solvent)
System I	t^1=0, 95%A; t^2=5, 95%A; t^3=52.5, 0%A; t^4=57.5, 0%A t^5=76.5, 95%A; re-condition until t^6=86.5
System II	t^1=0, 100%C; t^2=5, 100%C; t^3=10, 95%C; t^4=15, 95%C; t^5=20, 100%C; re-condition until t^6=25
System III	t^1=0, 100%C; t^2=5, 100%C; t^3=10, 90%C; t^4=15, 90%C; t^5=20, 100%C; re-condition until t^6=25

System I:- Water (A) and MeCN/water (B), both containing 0.1% TFA; System II:- Hexane (C) and acetonitrile (D); System III:- Hexane (C) and isopropanol (E).

change in polarity of the solvent system. System II was used under isocratic conditions with varying compositions

of C and E. After each composition change, the column was allowed to re-equilibrate. It should be noted that no optimisations of the chromatographic parameters, such as flow rate, solvent composition etc were undertaken in the limited time available at the end of this project. All columns were equilibrated with the System (I, II or III) over several hours, or until a stable base line was recorded. The test compounds were *(11)*, *(13)*, *(14)* and *(15)*, all samples were dissolved in ethanol.

The model column (*CSP 1*) was of the type shown in Fig 10.1, and was tested using System I under the conditions described. *CSP 1* Was initially tested using *(11)*. The peak at R_t=40.3min is the required peak. This was confirmed



CSP 1

Fig 10.1

by TLC analysis, by collection of the fraction eluted from the column and analysis with an authentic sample of *(11)*. It can be seen from the recorded chromatogram (**Fig 10.2**), that at the apex, there are two peaks of almost equal height. Injection of a blank sample, ethanol, and re-injection of *(11)* confirmed that this was not a characteristic of the column.

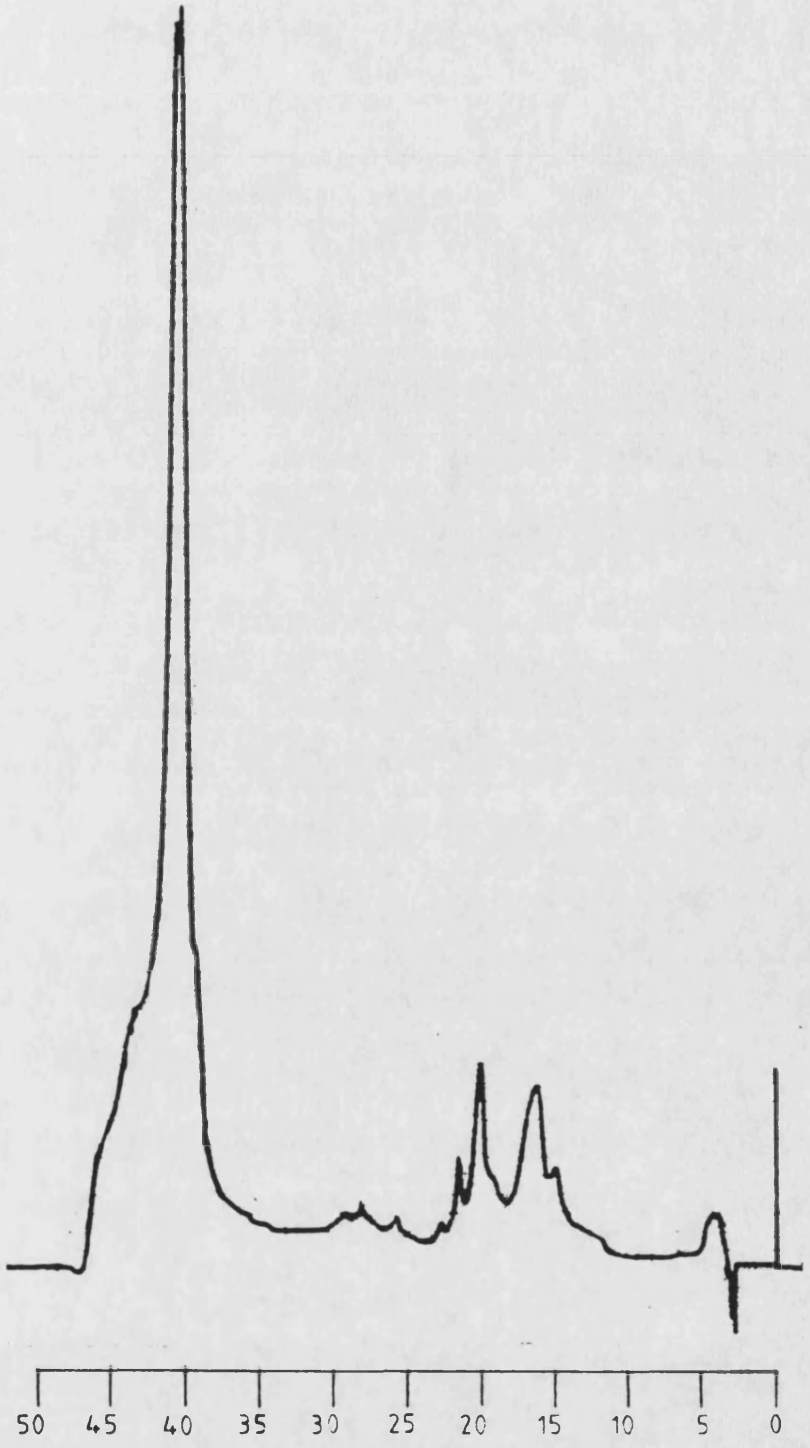


Fig 10.2. Qualitative HPLC analysis of *tert*-butyloxycarbonyl-L-leucine-D,L-(allyl)glycine methyl ester (*11*) on ***CSP 1*** using System I eluent under the described chromatographic conditions.

Using *CSP 1*, an attempt was made to resolve the two diastereomers of the cyclic peptides, (13) and *(14)*, using System I, the resultant chromatogram is presented (**Fig 10.3**). For comparative purposes the cyclic peptides were also injected onto a C_{18} column. The tabulated results (**Table 10.2**) show a small improvement in the resolution of the two cyclic peptides using *CSP 1* over that of the C_{18} column. There is also a considerable decrease in retention

Table 10.2. Comparison of retention times of cyclic peptides (13) and *(14)* on C_{18} and *CSP 1* columns using System I.

	(13)		(14)		
	R_t/min	k'	R_t/min	k'	α
C_{18}	20.38	10.65	22.38	11.79	1.11
CSP 1	9.13	2.84	6.75	1.84	1.54

time with a reversal of elution order. Däppen *et al*,[6] also observed a reversal of elution order, in his study of the separation of enantiomeric amine derivatives, in which the component that is first eluted is of opposite configuration to that of the chiral selector bonded to the stationary phase (**Fig 10.4**). The test compound used, under the condition described by Däppen *et al*,[6] was *R,S-N*-(1-phenylethyl)-3,5 dinitrobenzoylamide (**Fig 10.5**). The first peak eluted using CSP 21 was of *S*(+)-configuration, and for

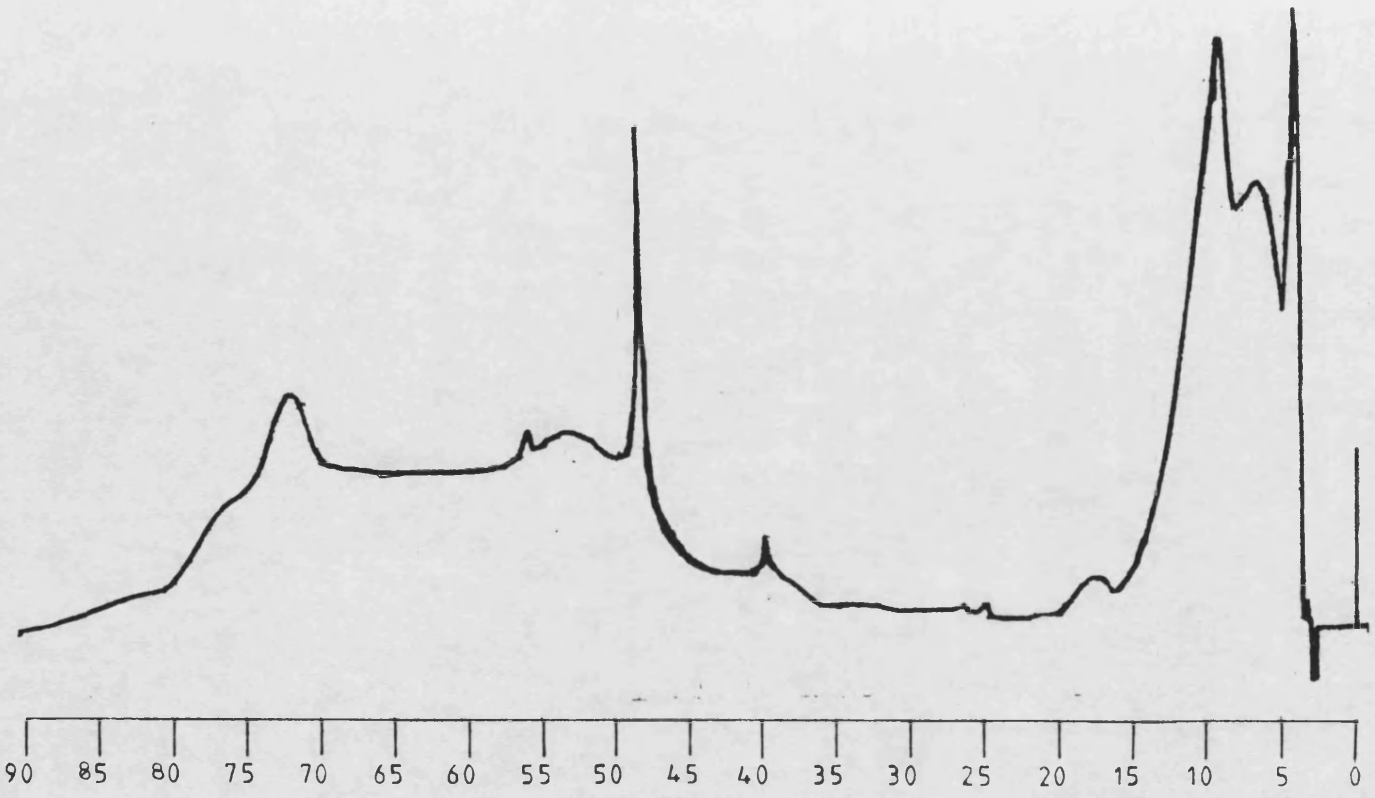
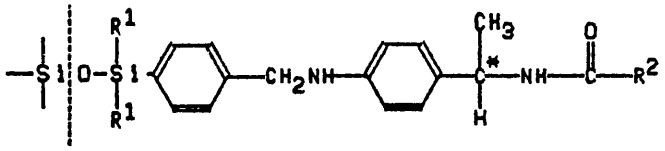


Fig 10.3. Qualitative HPLC analysis of cyclo-L-leucine-D,L-(allyl)glycine ***(13)*** and ***(14)*** on ***CSP 1*** using System I eluent under the described chromatographic conditions.

CSP 22 this was of R(-)-configuration. The small improvement in the separation factor (α) is, however, misleading, since it suggests increased baseline resolution of *(13)* and *(14)* on *CSP 1* over that of the C_{18} column.



CSP 21 * R-configuration; R^1=OCH_3, R^2=CH_3

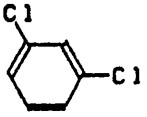
CSP 22 * S-configuration; R^1=CH_3, R^2= 

Fig 10.4

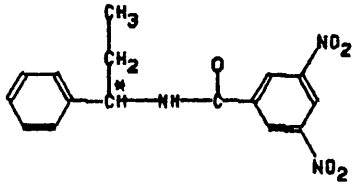


Fig 10.5

However, it can seen from the chromatogram (Fig 10.3) that no base line resolution is observed. This can be explained by the fact that the calculation of capacity ratio (k') values is peak to peak, and does not take into account such factors as trailing peaks. Hence, "realistic" α values, can only be calculated if the system has been optimised to achieve maximum resolution of the required components. Therefore, the α value calculated does not give a realistic

view of the resolution, but the result is still encouraging with regards to the reversal of the eluted components, since this suggests that a separation mechanism is taking place.

Further tests were performed on *CSP 2* and *(3)* (Fig 10.6) using System II. The test compound was a racemic mixture of *(16)* (Fig 10.7) which had retention times of 2.0 and 3.0min for *CSP 2* and *3* respectively. Chromatograms following injections of *(16)* onto *CSP 2* and *3* are presented (Fig 10.8 and 10.9), and show a major peak with a smaller shoulder. It seems unlikely that this signifies enantiomer resolution in view of the very different peak heights. However, the reason for the appearance of this second peak is not obvious.

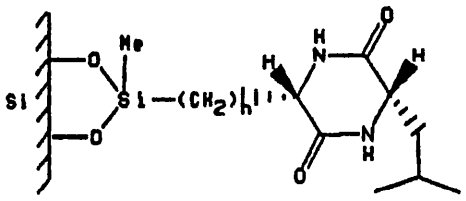


Fig 10.7. *CSP 2*, n=3; *CSP5*, n=5

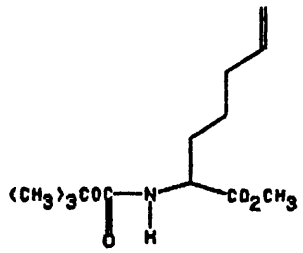


Fig 10.7

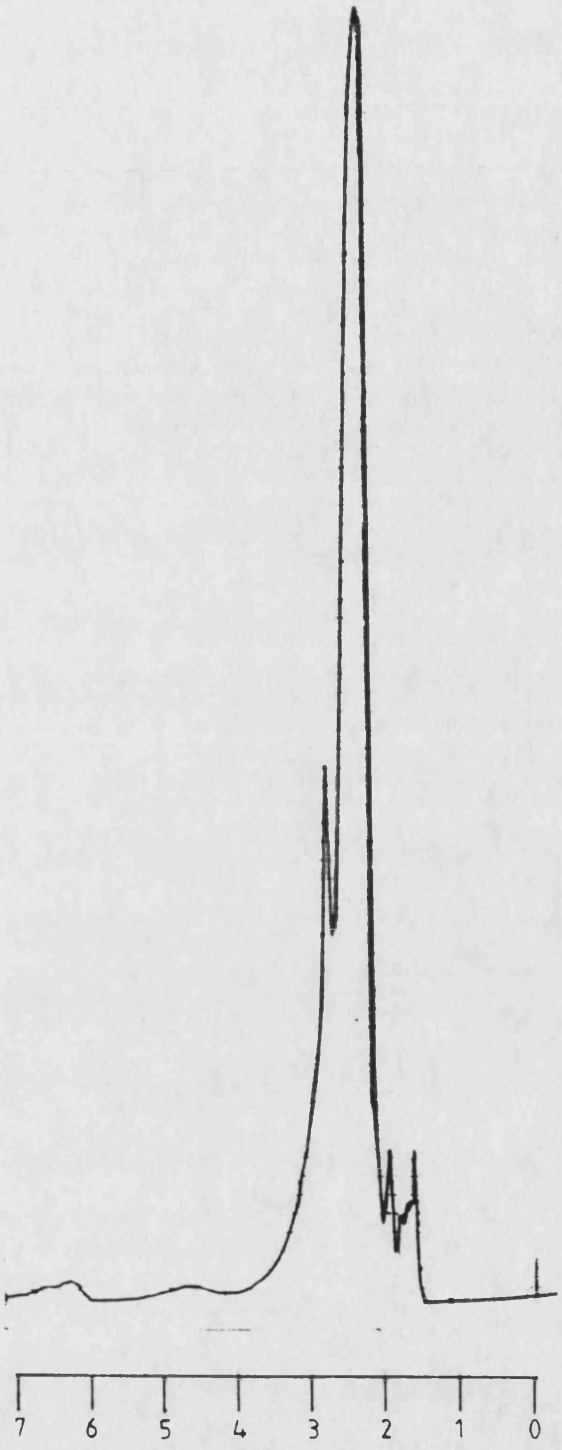


Fig 10.8. Qualitative HPLC analysis of *tert*-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester (*16*) on ***CSP*** *2* using System II eluent under the described chromatographic conditions.

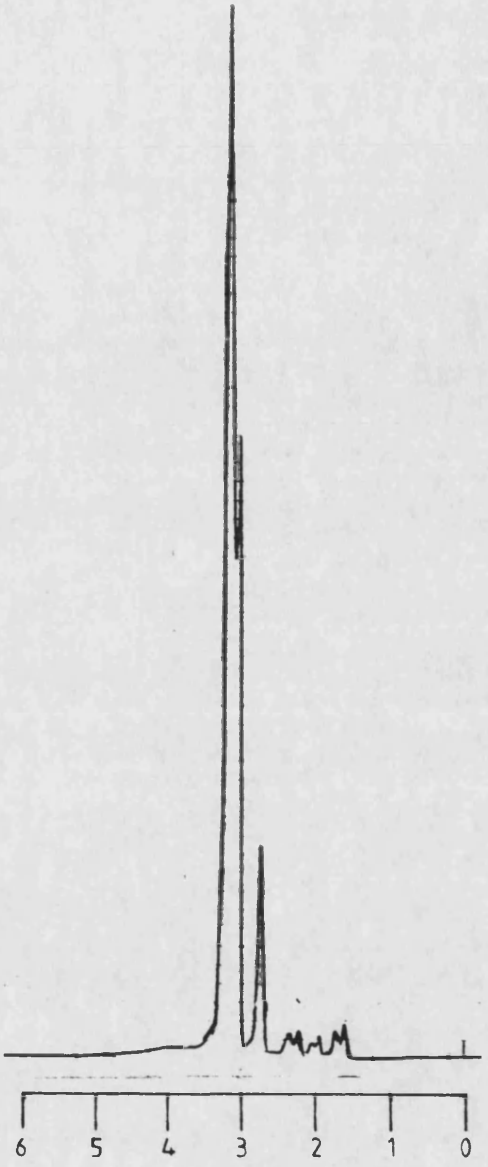


Fig 10.9. Qualitative HPLC analysis of tert-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester *(16)* on ***CSP*** *3* using System II eluent under the described chromatographic conditions.

Since (16) was eluted rather quickly using System II, an attempt was made to increase the retention time. Hence, the less polar solvent combination of isopropanol and hexane was employed (System III). The use of pure hexane caused an increase in retention time (Fig 10.10) (R_t=4.77min), but no separation was observed. Various mixtures of isopropanol/hexane were then investigated briefly (Table 10.3). The effect of increasing the isopropanol content served to produce a decrease in retention time, but in all cases no resolution was observed. From the chromatogram (Fig 10.10), two side peaks

Table 10.3. Retention time of (16) injected on *CSP* 3 using isopropanol/hexane mixtures under isocratic elution conditions.

% Isopropanol	Retention time/min
0	4.77
5	4.90
25	3.94
50	3.52
75	3.90

(R_t=4.41 and 5.37min) are visible either side of the main peak (R_t=4.77min). It is possible that these two side peaks are the result of a separation of the individual

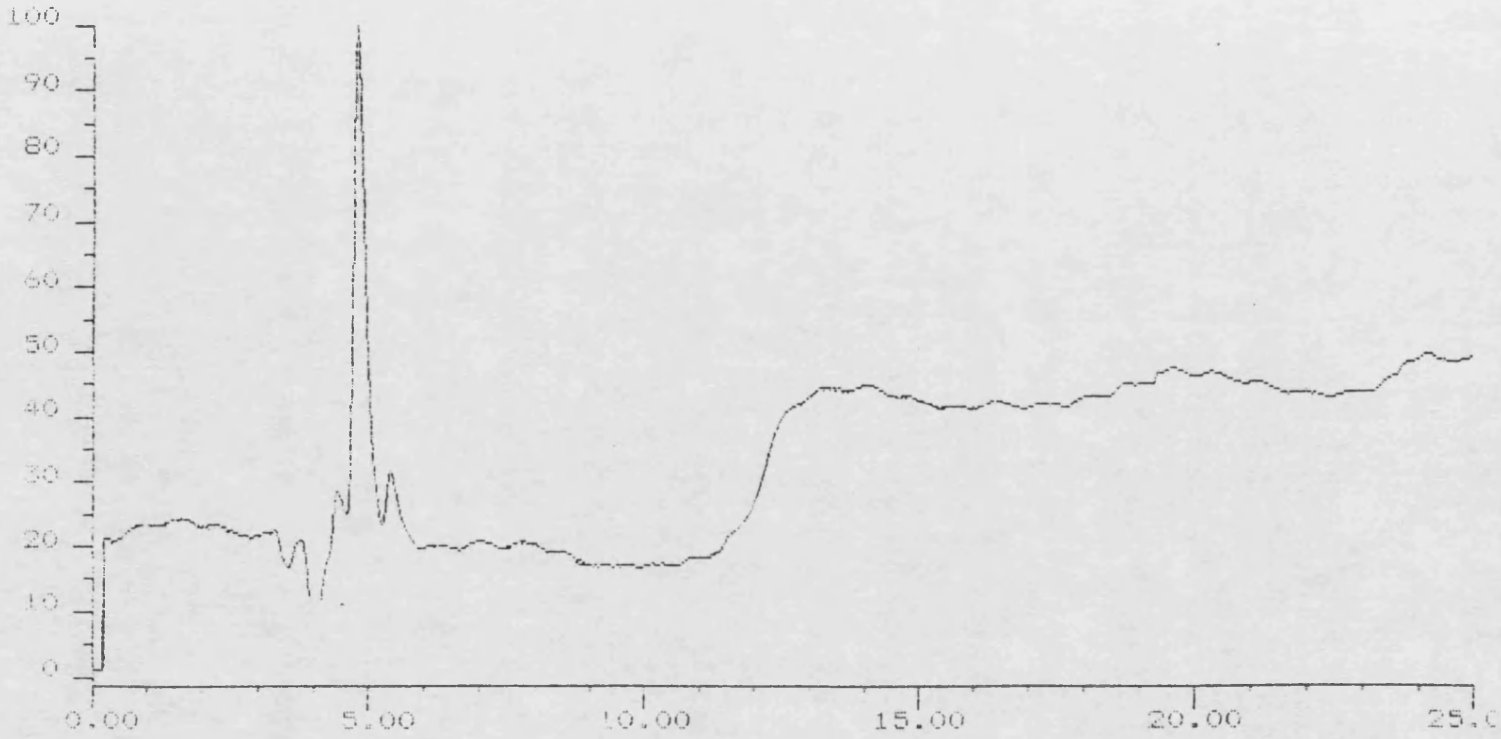


Fig 10.10. Qualitative HPLC analysis of tert-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester (*16*) on ***CSP*** 3 using hexane as the eluent.

enantiomers of the racemate (*15*). Unfortunately this could not be confirmed, as collection of the required eluting fractions was not possible without cross-contamination from the major peak. A gradient elution profile was also investigated briefly (**Table 10.1**). A chromatogram from these investigations is shown (**Fig 10.11**). A useful increase in retention time was observed (R_t=5.75min), but no resolution of the enantiomer of (*16*) could be observed. Again the two side peaks (R_t=4.91 and 6.83min) are visible, and much better resolved. However, confirmation of the identity of the two peaks again could not be made.

10.3 Conclusions.

It is evident that more work is required to determine whether resolution of enantiomers using cyclic peptides as the chiral selectors can be achieved. Initial results from the analysis of the two diastereomeric cyclic peptides, (13) and (*14*), are encouraging, as the elution order of the cyclic peptides was reversed when using *CSP 1*, indicating that a separation mechanism is operating. No observable resolution of the peptides (*11*), (*16*) were achieved with *CSP 1*, 2 or 3. This may reflect on incorrect choices of conditions or the fact that the columns used in this study were not end-capped, and therefore, it is likely that more than one separation mechanism may be operating, which may/may not have a cancelling effect on any resolution that may be achieved. It is probable that a ring enlargement of

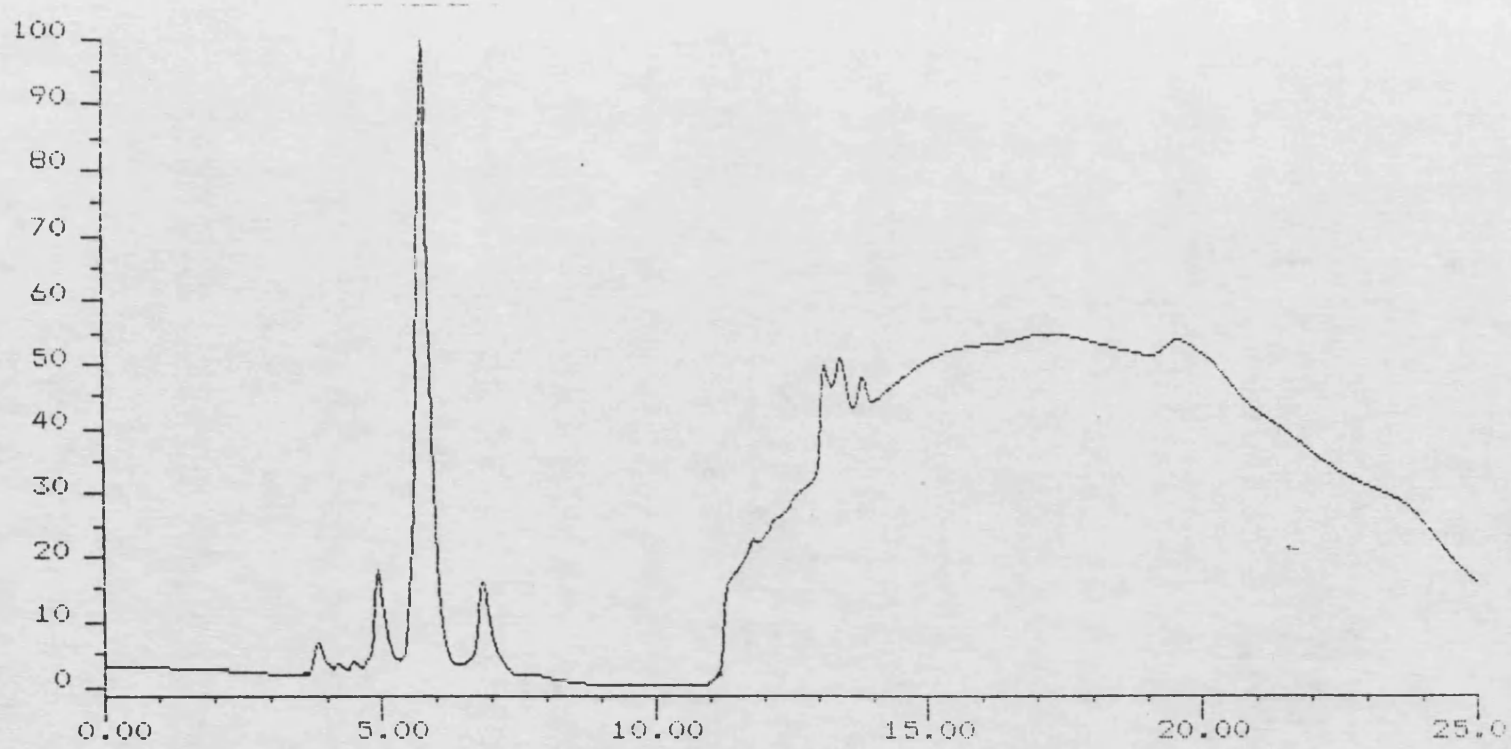


Fig 10.11. Qualitative HPLC analysis of *tert*-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester *(16)* on ***CSP 3*** using System III eluent under the described chromatographic conditions.

the active sites is necessary, providing more interactive sites for resolution to occur. It should also be borne in mind that the choice of substrate may also determine if a resolution is observed.

Optimisation of the solvent system will be necessary, since the appropriate conditions have not yet been established, and long term stability of the columns will also need to be investigated.

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4. K.B. Lipowitz, D.A. Demeter, R. Zerarra, R. Larter and T. Darden; J. Amer. Chem. Soc., 1988,110,3446.
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6. R. Däppen, V.R. Meyer and M. Arm; J. Chromatogr., 1984,295,367.
7. W.H. Pirkle, J.M. Finn, J.L. Schreiner and B.C. Hamper; J. Amer. Chem. Soc., 1981,103,3964.
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9. W.H. Pirkle and T.C. Pochapsky; J. Amer. Chem. Soc., 1986,51,352.
10. W.H. Pirkle and T.C. Pochapsky; J. Org. Chem., 1986,51,4991.

EXPERIMENTAL

CHAPTER ELEVEN.

EXPERIMENTAL.

11.1 Instrumentation and experimental technique.

Melting points (m.p.) were determined on commercially available apparatus (Electrothermal Mk III or Gallenkamp), and are uncorrected. Optical rotations were measured using a Perkin Elmer 141 Polarimeter fitted with a sodium lamp and a filter to isolate the sodium D line (589nm).

Infrared spectra were recorded in the range 4000-600cm^{-1} using a Perkin Elmer 1310 grating spectrometer and peaks are reported as (υ_{max}) wavenumbers (cm^{-1}). Spectra of liquid samples were recorded on thin films, and solid samples were mulled with nujol.

Proton magnetic (^{1}H N.M.R.) spectra were recorded at 270MHz on a JEOL GNM GX FT 270 spectrometer (as presented) unless otherwise stated, and on Hitachi Perkin Elmer High Resolution R-32B and Varian Anaspect EM-360 spectrometers. ^{13}C N.M.R. spectra were recorded on a JEOL GNM GX FT 270 spectrometer. ^{1}H and ^{13}C N.M.R. spectra were recorded in $CDCl_3$, unless otherwise stated, and are expressed in parts per million (p.p.m.) downfield from internal trimethylsilane (TMS). Multiplets are given as follows:- singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin), sextet (sex), heptet (h), multiplet (m) and broad (br).

Routine mass spectra from both electron impact (E.I.) and chemical ionisation (C.I.), and high resolution mass determinations were recorded with a VG Analytical 7070E instrument with a VG2000 data system. Unless otherwise stated, the data provided is that from C.I. using isobutane as reagent gas. E.I. spectra were produced with an ionising potential of 70eV. Where possible, the molecular ion peak M^+ or $[M+1]^+$ and the base peak are indicated, as are all sizeable fragmentations with assignments. Elemental analyses were carried out using a Carlo Erba 1106 Elemental Analyser.

For experimental procedure, a complete description is given, and details quantities of reagent, yield and characterisation details are included.

Thin layer chromatography (T.L.C.) was used extensively as a qualitative guide during reactions and for assessing purity of compounds. Whatman AL Sil G/UV or Merck DC-Alufolien Aluminiumoid 60 F_{254} sheets (dipped in triethylamine and air dried) containing fluorescent indicator were used for this purpose. Visualisation of reaction components was achieved by illumination under short wavelength (254nm) ultraviolet light when possible, plates were developed with 5%(w/v) aqueous solution of potassium permanganate ($KMnO_4$) and then washed in water or a 7%(w/v) methanol solution of *dodeca*-molybdophosphoric acid (PMA), followed by warming of the T.L.C. plate.

Unless otherwise stated, petroleum refers to petroleum spirit with a boiling point range 60-80^0 and which was

distilled before use as eluant in column chromatography.

Medium pressure flash column chromatography was routinely employed using Amicon Matrex Silica Si Chromatography Medium (particle size 35-70μm). A pressure gradient was developed using a small, commercially available hand bellow (Gallenkamp). Columns were prepared in the eluting or the least polar solvent, and then eluted isocratically or with solvent mixtures of steadily increasing polarity. Materials that were pre-adsorbed onto the chromatography support were applied as a thin layer to the top of the column.

Tetrahydrofuran (THF) was pre-dried over sodium wire, then refluxed over sodium benzophenone ketyl under dry nitrogen until anhydrous. This was redistilled immediately prior to use.

Glassware used for water sensitive reactions was baked in an oven at 120^0 for 12h and allowed to cool in a desiccator over $CaCl_2$. Flasks and stirring bars were additionally flame dried under nitrogen.

In all experiments, the excess solvent was evaporated with a Büchi rotary evaporator using a water aspirator at room temperature or in a water bath at a temperature of $<40^0C$ to avoid unnecessary heating. All yields quoted are for the purified products, and are uncorrected.

All other general reagents and solvents were purified and dried when required using the methods described in A.I. Vogel "Textbook of Practical Organic Chemistry" 4^{th} Edition, Longman, London and New York 1978.

2D Homonuclear shift correlated spectra for δ_H couplings using the C.O.S.Y. microprogram have been used extensively throughout this project when proton assignments were unclear. The lettering system used for the alkenyl side chain of the glycine residue is as indicated in Fig 11.1.

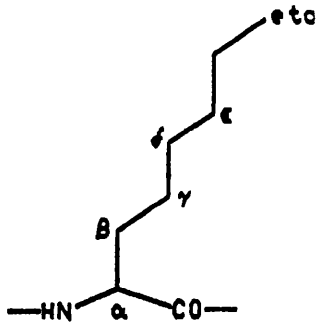

Fig 11.1

The numbering system used to denote the position of the chiral carbon when more than one chiral centre is present is as shown in Fig 11.2.

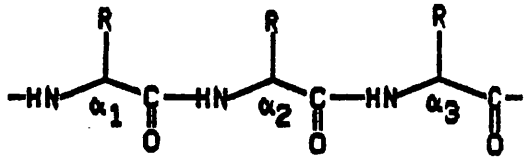

Fig 11.2

11.2 Experimental Procedure.

Synthesis of tert-butyloxycarbonyl-L-leucine.[Lit 1] *(1)*

N_2H-Leu-OH ⟶ Boc-Leu-OH (*1*)

A suspension of L-Leucine (3.0g, 0.023M) in dioxane/H_2O ($70cm^3$, 2:1v/v), was treated with Na_2CO_3, (2N, $45cm^3$) added, and then cooled to 0°C when $(Boc)_2O$ (5.74g, 0.026M, 1.13eq) was added. The reaction mixture was stirred and allowed to warm to R.T. overnight. Dioxane was removed *in vacuo* and H_2O ($100cm^3$) was added to dissolve the suspension. The aqueous layer was washed with EtOAc ($300cm^3$) and cooled to 0°C when citric acid (1N, $75cm^3$) was added to the aqueous phase to precipitate the product. The product was recrystallised from MeOH/H_2O and dried over silica gel (self-indicating) to give the product (*1*) as the mono-hydrate (4.80g, 84%).

m.p. 87-88°C; [Lit[1] 78-81°C; Lit[2] 67-72°C (mono-hydrate)].

$[\alpha]_D$= -22.1° (c=1, acetic acid); [Lit[2] $[\alpha]_D$= -24° (c=2.002, acetic acid)].

υ_{max}(nujol); 3440 (OH, water of recrystallisation), 3250 (NH str), 1665 (C=O str), 1240cm^{-1} (C-O str).

$\delta_H(CDCl_3)$; 0.96[6H, d, $(CH_3)_2$, J=6.5Hz], 1.45[9H, s, $(CH_3)_3$],

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Lit [1]:- P. Schwyzer, P. Sieber and H. Kappeler; *Helv. Chim. Acta.*,1959,281,2622.

Lit [2]:- J.C. Sheenan and G.P. Hess; *J. Amer. Chem. Soc.*, 1955,77,1067.

1.50-1.72[3H, brm, CH, CH_2], 4.32[1H, brm, αCH], 4.95[1H, brd, NH urethane, J=8.0Hz].

m/e (C.I.); 232[$(M+1)_+$, 8%], 176[$(M+1)^+-(CH_3)_3C$, 100], 130[176-CO_2, 71], 86[130-CO_2, 27].

Found:- C,53.5; H,9.55; N,5.62. $C_{11}H_{21}NO_4$ requires C,53.2; H,9.24; N,5.62%.

Synthesis of L-leucine methyl ester hydrochloride. (2)

N_2H-Leu-OH ⟶ HCl.H_2N-Leu-OMe **(2)**

Method 1.[Lit 3]

To leucine (1.0g, 7.6mM) was added 2,2 dimethoxypropane (100cm^3) and conc. HCl (7.5cm^3), and the reaction mixture stirred at R.T. for 48h. The volatile components were then removed *in vacuo* at a water bath temperature not exceeding 50°C, and the residue dissolved in the minimum volume of dry MeOH. Addition of dry ether (*ca* 250cm^3) precipitated the crude product *(2)*, which was filtered and re-crystallised from MeOH/ether (0.9466g, 68%).

m.p. 148-149°C; (Lit[3] 146-148; Lit[4] 147-148°C).

$[\alpha]_D$= +13.4 (c=1, H_2O); [Lit[4] $[\alpha]_D$= +13.4 (c=5.075, H_2O)].

υ_{max}(nujol); 1730 (C=O str), 1250, 1220cm^{-1} (C-O str).

Lit [3]:- J.R. Rachele; *J. Org. Chem.*, 1963,28,2898.

Lit [4]:- M.F. Scholl, J.B. Larkin, L.B. Rochland and M.S. Dunn; *J. Org. Chem.*, 1947,12,490.

60MHz, $\delta_H(CDCl_3)$; 1.0[6H, $(CH_3)_2$], 1.9[3H, CH, CH_2], 3.8[3H, OCH_3], 4.2[1H, αCH].

m/e (E.I.); 146[$(M+1)^+$, 100%), 86[M+1-CO_2CH_3, 92].

Method 2.[Lit 4]

Dry HCl gas was bubbled through a suspension of leucine (20g, 0.15M) in dry MeOH (120cm^3) until a sample of the reaction mixture gave one spot by T.L.C. (solvent system; butanol:AcOH:H_2O; 4:1:1:v/v; ninhydrin). The solvent was removed *in vacuo* until constant weight was achieved. The product was recrystallised from MeOH/ether and dried over P_2O_5 *in vacuo* (24.13g, 87%).

m.p. 147.5-148°C; (Lit[4] 147-148°C).

$[\alpha]_D$= +13.6 (c=1,H_2O); [Lit[4] $[\alpha]_D$= +13.4 (c=5.075, H_2O)].

υ_{max}(nujol); 1730 (C=O str), 1240, 1220cm^{-1} (C-O str).

$\delta_H(CDCl_3)$; 0.95[6H, q, $(CH_3)_2$, J=2.5Hz], 1.74[2H, brm, CH_2], 1.85[1H, brm, CH], 3.84[3H, s, OCH_3], 4.15 [1H, t, αCH, J=7.0Hz].

m/e (C.I.); 146[$(M+1)^+$, 100%], 86[$(M+1)^+$-CO_2CH_3, 96].

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucine methyl ester. (3)

Boc-Leu-OH + HCl.N_2H-Leu-OMe ⟶ Boc-Leu_2-OMe (3)

Dicyclohexylcarbodimide method.[Lit 2,5]

To a cooled (0°C) solution of (1) (71mg, 0.31mM, 1.1eq) HOB^t (45mg, 0.33mM) and DCCI (64mg, 0.31mM) in DCM (5cm^3), was added the free amino component of (2), prepared by dissolving the HCl salt (50mg, 0.28mM) in DCM (5cm^3), adding diisopropylamine (0.05cm^3) and cooling to 0°C. The reaction mixture was stirred at 0°C for 3h and then at R.T. overnight. The reaction mixture was filtered and the filter cake thoroughly washed with EtOAC (15cm^3). The organic layer was washed with citric acid (1N), Na_2CO_3 (1N), saturated brine, dried over $MgSO_4$ and the solvent removed in vacuo to leave an oil. The oil was chromatographed on silica gel (DCM/MeOH, 4:1v/v) and the product (3) recovered as a white solid (28mg, 28%).

m.p. 100-105°C; (Lit[6] 132-133°C; Lit[7] 136-137°C; Lit[8] 141-142°C).

Lit [5]:- W. Konig and R. Geiger; *Chem. Ber.*, 1970,103,788.

Lit [6]:- D.E. Nitechi, B. Halpern and J.W. Westley, *J. Org. Chem.*, 1964,33,869.

Lit [7]:- K. Suzuki, Y. Saski, N. Endo and Y. Mikara; *Chem. Pharm. Bull.*, 1981,29,233.

Lit [8]:- J.E. Shields, S.T. McDowell, J. Pavlos and G.R. Gray; *J. Amer. Chem. Soc.*, 1968,90,3549.

$[\alpha]_D$= -26 (c=1, MeOH); [Lit[6] $[\alpha]_D$= -50.4 (c=1, MeOH); Lit[7] $[\alpha]_D$= -55.0 c=1, MeOH); Lit[8] $[\alpha]_D$-25.7 (c=0.3, MeOH)].
$\delta_H(CDCl_3)$; 0.94[12H, dt, 2X$(CH_3)_2$, J=6.5Hz], 1.44[9H, s, $(CH_3)_3O$], 1.50-1.66[6H, brm, CH, CH_2], 3.73[3H, s, OCH_3], 4.11[1H, brm, αCH], 4.61[1H, dt, α_2CH, J=8.5Hz], 4.90[1H, brd, NH urethane, J=8.0Hz], 6.50[1H, brd, NH amide, J=8.0Hz].

Mixed anhydride method.[Lit 9,10,11]

Boc-Leu-OH + HCl.N_2H-Leu-OMe ⟶ Boc-Leu_2-OMe (3)

Method 1.

The free amino component was formed by dissolving (2) (1.12g, 4.8mM) in DMF (5cm^3) with warming, adding TEA (0.36cm^3, 4.8mM) and cooling to -15°C. (1) (1.1g, 4.8mM) was dissolved in THF (14cm^3), NMM (0.53cm^3, 0.48mM) added, and the solution cooled to -15°C when IBC (0.63cm^3, 0.48mM) was added. After ca 1min the free amino component was added slowly . The reaction mixture was stirred and allowed to warm to R.T. overnight. The reaction mixture was filtered and the filter cake thoroughly washed with EtOAc. The volume of the filtrate was reduced until the liquid became

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Lit [9]:- J.R. Vaughan Jr. and R.L. Osato; J. Amer. Chem. Soc., 1951,73,3547.

Lit [10]:- T. Wieland and M. Bernhard; Ann. Chem., 1951,572,190.

Lit [11]:- R.A. Boissonnas; Helv. Chim. Acta., 1951,34,874.

oily, and the product precipitated with H_2O (50cm^3), dried over P_2O_5 before recrystallising with MeOH/H_2O 1.37g, 80%, m.p. 138-139°C).

$[\alpha]_D$= -49.8 (c=1, MeOH).

υ_{max}(nujol); 3360, 3270 (NH str), 1760, 1680, 1650 (C=O str), 1550cm^{-1}.

$\delta_H(CDCl_3)$; 0.93[12H, dt, 2X$(CH_3)_2$, J=6.0Hz], 1.44[9H, s, $(CH_3)_3CO$], 1.48-1.70[6H, brm, CH, CH_2], 3.73[3H, s, OCH_3], 4.10[1H, brm, α_1CH], 4.63[1H, dt, α_2CH, J=8.5Hz], 4.88[1H, brd, NH urethane, J=7.5Hz], 6.46[1H, brd, NH amide, J=8.0Hz].

m/e (C.I.); 359[$(M+1)^+$, 71%], 303[$(M+1)^+$-$(CH_3)_3C$, 94], 254[303-CO_2, 41], 186[$(M+1)^+$-$CONHC_5H_{10}CO_2CH_3$, 24], 130[186-C_4H_8, 57], 86[130-CO_2, 100].

Method 2.

(1) (9.24g, 0.041m) Was dissolved in DMF (40cm^3), NMM (4.5cm^3, 0.041M) added and the mixture cooled to -15°C when IBC (5.5cm^3, 0.041M) was added. The free amino component was formed by dissolving *(2)* (7.78g, 0.043M, 1.05eq) in DMF 9120cm^3), NMM (4.7cm^3, 0.043M) added, cooled to -15°C and then added to the cooled solution of *(1)*. The reaction mixture was stirred at -15°C for 6h and filtered, the filter cake being thoroughly washed with EtOAC. The volume of the filtrate was reduced to a light oil consistency and H_2O (*ca* 300cm^3) was added to precipitate the product. The product was recrystallised from MeOH/H_2O and dried over P_2O_5 *in vacuo*

(12.85g, 88%, m.p. 138-139°C), and found to be identical to the product (3). synthesised by the method described above (Method 1).

Deprotection of tert-butyloxycarbonyl-L-leucyl-L-leucine methyl ester. [Lit 12]

$$\text{Boc-Leu}_2\text{-OMe} \longrightarrow \text{TFA}^-\text{H}_3\text{N}^+\text{-Leu}_2\text{-OMe}$$

A solution of (3) (0.72g, 2mM) in TFA (6.0cm^3) was allowed to stand at R.T. for 1h while protected by a $CaCl_2$ drying tube. The solvent was removed *in vacuo* leaving an oil. The de-protected di-peptide was precipitated from ether/pet ether and used without characterisation.

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucine methyl ester. [Lit 8] ***(4)***

$$\text{Boc-Leu-OH} + \text{H}_2\text{N-Leu}_2\text{-OMe} \longrightarrow \text{Boc-Leu}_3\text{-OMe (4)}$$

Boc-Leu_3-OMe (4) Was prepared from (1) and H_2N-Leu_2-OMe (from treatment of (3) with TFA), by a similar method to that used for the preparation of the di-peptide (3). The product (4) was isolated in 72% yield.

m.p. 157-159°C; (Lit[8] 156-159°C).

Lit [12]:- H. Kappeler and R. Schwyzer; *Helv. Chim. Acta.*, 1960,43,1453.

$[\alpha]_D$= -64.3 (c=1, MeOH); [Lit[8] $[\alpha]_D$= -21.7 (c=0.4, MeOH)].

υ_{max}(nujol); 3290 (NH str), 1745 (C=O str), 1680, 1660, 1630 (C-O str), 1530cm^{-1}.

$\delta_H(CDCl_3)$; 0.92[18H, m, 3X$(CH_3)_2$], 1.44[9H, s, $(CH_3)_3CO$], 1.46-1.75[9H, brm, CH, CH_2], 3.72[3H, s, OCH_3], 4.32[1H, brm, α_1CH], 4.48[2H, brm, α_2,α_3CH], 5.21[1H, brd, NH urethane, J=6.5Hz], 6.69[1H, brd, NH-α_2CH amide, J=8.0Hz], 6.79[1H, brd, NH-α_3CH amide, J=8.0Hz].

m/e (C.I.); 472[$(M+1)^+$, 15%], 416[$(M+1)^+-(CH_3)_3$, 30], 372[416-CO_2, 12].

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucyl-L-leucyl-L-leucine methyl ester.[Lit 8] (5)

Boc-Leu-OH + H_2N-Leu_3-OMe ⟶ Boc-Leu_4-OMe (5)

Boc-Leu_4-OMe (5) Was prepared from (1) and H_2N-Leu_3-OMe (from treatment of (4) with TFA), by a similar method to that used for the preparation of the di-peptide (3). The product (5) was isolated in 41% yield.

m.p. 200-201°C; (Lit[8] 207-208°C).

$[\alpha]_D$= -86.9 (c=1, MeOH). [Lit[8] $[\alpha]_D$= -16.4 (c=0.25, trifluoroethanol),

$\delta_H(CDCl_3)$; 0.92[24H, brs, 4X$(CH_3)_2$], 1.43[9H, s, $(CH_3)_3CO$], 1.50-1.75[12H, brm, CH, CH_2], 3.71[3H, s, OCH_3], 4.35[1H, brm, α_1CH], 4.45-4.80[3H, brm, $\alpha_2,\alpha_3,\alpha_4$CH], 5.64[1H, brd, NH urethane], 7.58[3H, brm, NH-$\alpha_2,\alpha_3,\alpha_4$CH amide].

m/e (C.I.); 585[$(M+1)^+$, 18%], 529[$(M+1)^+-(CH_3)_3C$, 10], 485[529-CO_2, 29].

Synthesis of benzylidine glycine methyl ester.[Lit 13] *(6)*

$$H_2NCH_2CO_2H \longrightarrow PhCH=NCH_2CO_2CH_3 \quad (6)$$

To a suspension of HCl-Gly-OMe (10g, 0.08M) in DCM (160cm^3) was added benzaldehyde (8.8cm^3, 0.08M, 1.0eq), TEA (11.8cm^3, 0.16M) and $MgSO_4$ (8.0g). The reaction mixture was stirred at R.T. for 48h while protected by a $CaCl_2$ drying tube. The reaction mixture was filtered and the solvent removed *in vacuo*. To the crude product was added H_2O (100cm^3), ether (100cm^3) as an extractant. The aqueous layer was further extracted with another litre of ether. The organic layer was washed with saturated brine (200cm^3), H_2O(200cm^3), dried over $MgSO_4$ and the solvent removed *in vacuo* to give the product *(6)* as lemon-coloured oil (11.72g, 83%).

υ_{max}(liquid film); 3040, 3010 (Ar-H, C=C str), 1720 (C=O str), 1625 (C=C str), 1420 (-CH= str), 1230 (C-O str), 725-680cm^{-1} (5 adjacent H, mono substituted Ar ring).
$\delta_H(CDCl_3)$; 3.76[3H, s, OCH_3], 4.41[2H, d, CH_2], 7.42, 7.77[5H, m, C_6H_5], 8.28[1H, s, -CH=].

Lit [13]:- G.S. Stork, A.Y.W. Leong and A.M. Touzin; J. Org. Chem., 1976,41,3491.

Synthesis of D,L-(allyl)glycine methyl ester.[Lit 14] *(7)*

$PhCH{=}NCH_2CO_2CH_3$ ⟶ $HCl.H_2N$-(allyl)Gly-OMe *(7)*

+

$HCl.H_2N$-$(allyl)_2$Gly-OMe *(8)*

(6) (11.72g, 0.066M) Was dissolved in THF (130cm^3) and cooled to -78°C. The temperature of the reaction was monitored with a thermometer inside the reaction flask. A suspension of $KOBu^t$ (sublimed), in THF (105cm^3) was slowly added dropwise, keeping the temperature of the reaction mixture below -70°C. The dark red solution was stirred for a further 0.5h after complete addition of $KOBu^t$, when a solution of allyl bromide (11.2cm^3, 0.066M, 1.0eq) in THF (75cm^3) was slowly added dropwise, keeping the temperature of the reaction below -70°C. The reaction mixture turned slowly from red to yellow at which stage it was allowed to warm slowly to -10°C and kept at -10°C for *ca* 12h. The reaction mixture was washed with saturated NH_4Cl solution (300cm^3) and separated. The aqueous layer was further extracted with ether (450cm^3) and the organic layers combined. The organic layer was washed with saturated NH_4Cl solution (300cm^3), H_2O (300cm^3), then dried over $MgSO_4$, and the solvent removed *in vacuo* to leave a yellow oil. The oil was dissolved in aqueous MeOH (90% MeOH, 150cm^3) and HCl gas bubbled through the solution. The solvent was removed *in vacuo* and the oil dissolved in H_2O (150cm^3), washed with ether (300cm^3) and the H_2O removed *in vacuo* to give a thick

oil (6.53g, 60%).

The yield of the mono-alkylated product (7) was determined by N.M.R. analysis by comparison of the two OCH_3 peaks with the αCH peak height. The presence of the di-alkylated product (8) was confirmed by M.S. analysis.

Analytical data for mono-alkylated product (7):-

$\delta_H(D_2O)$; 3.85[3H, s, OCH_3], 4.28[1H, q, αCH, J=5.0Hz], 5.33[2H, tm, $=CH_2-$, J=10.0 (trans), 8.0 (cis), 1.5Hz (gem)], 5.77[1H, m, -CH=].

m/e (C.I.); 130[$(M+1)^+$, 100%], 88[$(M+1)^+-C_3H_5$, 64], 70[130-CO_2CH_3, 51].

Analytical data for di-alkylated product (8):-

$\delta_H(D_2O)$; 3.88[3H, s, OCH3].

m/e (C.I.); 170[$(M+1)^+$, 28%]

Synthesis of tert-butyloxycarbonyl-D,L-(allyl)glycine methyl ester.[lit 1] *(9)*

$HCl.H_2N$-(allyl)Gly-OMe ⟶ Boc-(allyl)Gly-OMe (9)

(7) (1.31g, 7.9mM) Was dissolved in dioxane/H_2O (72cm^3, 2:1v/v) and cooled to 0°C when Na_2CO_3 (2N, 32cm^3) was added, followed by $(Boc)_2O$ (3.49g, 16.0mM, 2.0eq). The reaction mixture was stirred at R.T. overnight. Dioxane was removed in vacuo and H_2O was added to give a total volume of ca 100cm^3. The aqueous layer was extracted with EtOAc (150cm^3).

The extract was dried over $MgSO_4$ and the solvent removed *in vacuo* to afford the product *(9)* as an oil (1.54g, 83%).

υ_{max}(liquid film); 3340 (NH str), 2960 (C-H str), 1780,1700 (C=O str), 1480, 1360 (CH def), 1150cm^{-1} (C-O str).
$\delta_H(CDCl_3)$; 1.44[9H, s, $(CH_3)_3$], 2.49[1H, m, $-CH_2$], 3.74[3H, s, OCH_3], 4.36[1H, m, αCH], 5.14[2H, tm, $=CH_2$, J=11.0 (trans, 5.0Hz (cis)], 5.20[1H, d, NH urethane, J=8.0Hz], 5.70[1H, brm, -CH=].
$\delta_C(CDCl_3)$; 28.02 [$(CH_3)_3$], 36.46, 36.59[CH_2], 51.86[OCH_3], 52.71[αCH], 84.78[-CO-], 118.65[$=CH_2$], 132.21[-CH=], 154.97[C=O urethane], 172.26[C=O ester].
m/e (C.I.); 230[$(M+1)^+$, 55%], 174[$(M+1)^+-(CH_3)_3CO$, 100], 130[$(M+1)^+-(CH_3)_3COCO$, 74].

Synthesis of tert-butyloxycarbonyl-D,L-(allyl)-glycine.[Lit 14] ***(10)***

Boc-NH-(allyl)Gly-OMe ⟶ Boc-(allyl)Gly-OH *(10)*

(9) (0.2789g, 1.22mM) Was dissolved in MeOH (3.0cm^3) and cooled to 0°C when aqueous NaOH (1N, 1.8cm^3) was slowly added dropwise. The reaction mixture was kept at 0°C for a further 3h after the complete addition of base. The reaction mixture was acidified with citric acid (1N, 6.0cm^3). MeOH was removed *in vacuo* and H_2O (10cm^3) added. The

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Lit [14]:- B. Iselin, M. Fuerer and R. Schwyzer; *Helv. Chim. Acta.*, 1955,38,1508.

aqueous layer was extracted with EtOAc ($30cm^3$), the extract dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was chromatographed on silica gel (DCM/MeOH, 19:1v/v) to afford the product ***(10)*** as an oil (0.1205g, 46%).

υ_{max}(nujol); *ca* 3600-2800 (OH str), 2940 9NH str), 3060, 3020 (unsaturated str), 1760 (C=O str), 1550, 1440,1410 (C-H def), $1190cm^{-1}$ (C-O str).
$\delta_H(CD_3OD)$; 1.44[9H, s, $(CH_3)_3$], 2.43[2H, m, $-CH_2-$], 2.57[2H, m], 4.17[1H, dq, αCH, *J*=7.5, 3.0Hz], 5.10[1H, dt, $=CH_2$, *J*=17.0 (trans), 9.5 (cis), 2.0Hz (gem)], 5.80[1H, m, -CH=].

Synthesis of tert-butyloxycarbonyl-L-leucine-D,L-(allyl)glycine methyl ester. (11)

$HCl.H_2N$-(allyl)Gly-OMe + Boc-Leu-OH ⟶

Boc-Leu-(allyl)Gly-OMe ***(11)***

Method 1; Mixed anhydride method.[Lit 9]

(7) (0.7379g, 4.46mM) Was dissolved in DMF ($6cm^3$), TEA ($0.62cm^3$, 4.46mM) was added and the mixture cooled to -20°C. (1) (1.0336g, 0.447mM, 1.0eq) was dissolved in dmf ($4cm^3$), NMM ($0.49cm^3$, 4.47mM) added and the mixture cooled to -20°C when IBC ($0.53cm^3$, 4.47mM) was added. The reaction mixture was stirred at -20°C for *ca* 5min after which the free amino component was added, and the mixture was stirred at -20°C

for 6h and then at R.T. overnight. DMF was removed in vacuo and H_2O (75cm^3) was added. The aqueous layer was extracted with EtOAc (150cm^3), the extract dried over $MgSO_4$ and the solvent removed in vacuo. The crude product was chromatographed on silica gel (EtOAc/pet ether, 1:20-> 3:10v/v) to afford the product *(11)* as a waxy solid (0.7466g, 49%).

υ_{max}(nujol); 3320, 3260 (NH str), 3060 (C=C str), 1750, 1680, 1650 (C=O str), 1520, 1270, 1240, 1170cm^{-1} (C-O str).
$\delta_H(CDCl_3)$; 0.93[6H, dt, $(CH_3)_2$ leu, J=6.0Hz], 1.44[9H, s, $(CH_3)_3$], 1.51[1H, m, CH], 1.66[2H, m, CH_2], 2.48[2H, brm, CH_2], 3.72(8), 3.37(4)[3H, sX2, OCH_3X2], 4.22[1H, brm, α_1CH leu], 4.65[1H, m, α_2CH gly], 5.11[2H, tm, $=CH_2$, J=16.0 (trams), 7.5 (cis), 3.0Hz (gem)], 5.41[1H, brd, NH urethane, J=7.5Hz], 5.69[1H, m, -CH=], 7.11[1H, brd, NH amide].
m/e (C.I.); 343[$(M+1)^+$, 62%], 287[$(M+1)^+$-$(CH_3)_3C$, 100], 269[$(M+1)^+$-$CH_3)_3CO$, 8], 243[M+1-$(CH_3)_3COCO$, 66] 186[22], 130[54], 86[89].
Found:- C,59.8; H,9.06; N,7.98. $C_{17}H_{30}N_2O_5$ requires:- C,59.7; H,8.77; N,8.19%.

Synthesis of tert-butyloxycarbonyl-L-leucine-para-nitrophenyl ester.[lit 15] *(12)*

Boc-Leu-OH + pNP ——→ Boc-Leu-OpNP *(12)*

(1) (4.20g, 0.018M) And pNP (2.50g, 0.018M, 1.0eq) were dissolved in distilled EtOAc (35cm^3) and cooled to 0°C when DCCI (3.71g, 0.018M), in EtOAc (4cm^3) was added. The reaction mixture was stirred at 0°C for a further 30min and then at R.T. overnight while wrapped in aluminium foil to minimise exposure to light. The reaction mixture was cooled to 0°C again and DCU was filtered off and the solid thoroughly washed with EtOAc. The washings and filtrate were combined and the solvent removed *in vacuo* to leave a crystalline solid. The solid *(12)* was re-crystallised from pet ether (3.82g, 60%).

m.p. 93-93.5°C; (Lit[15] 83-84°C)

υ_{max}(nujol); 3290 (NH str), 1745, 1660 (C=O str), 1505, 1340cm^{-1} (NO_2 asym and sym str).

$\delta_H(CDCl_3)$; 1.02[6H, dd, $(CH_3)_2$, J=5.0, 1.0Hz], 1.47[9H, s, $(CH_3)_3$], 1.85[3H, m, CH, CH_2], 4.53[1H, m, αCH], 4.96[1H, brd, NH urethane, J=7.0Hz], 7.30, 8,28[5H, m, Ar-H].

m/e (C.I.); 353[$(M+1)^+$, 0.9%], 337[1.4], 297[$(M+1)^+-(CH_3)_3C$, 21], 253[$(M+1)^+-CO_2$, 8], 158[57], 140[100], 130[253-$C_6H_4NO_2$, 89], 86[130-CO_2, 61].

Lit [15]:- K. Vogler, R. Stader, P. Lanz, W. Leiger and E. Bohni; *Helv. Chim. Acta.*, 1965,126,1161.

Synthesis of tert-butyloxycarbonyl-L-leucine-D,L-(allyl)glycine methyl ester. (11)

$HCl.H_2N$-(allyl)Gly-OMe + Boc-Leu-OpNP ——→

Boc-Leu-(allyl)Gly-OMe *(11)*

Method 2; Active ester method.[Lit 16] *(11)*

(7) (0.57g, 3.4mM) Was dissolved in DMF ($10cm^3$), TEA ($0.8cm^3$, 5.9mM) was added and the mixture stirred at R.T. for *ca* 15min. This mixture was added *(12)* (2.10g, 6.0mM, 1.8eq) in DMF ($10cm^3$) and HOBt (0.58g, 43mM) in DMF ($2cm^3$). The reaction vessel was covered in aluminium foil and the contents stirred for 48h at R.T. DMF was removed *in vacuo* and EtOAc ($80cm^3$) was added. The organic layer was washed with citric acid (1N, $80cm^3$), Na_2CO_3 (1N, $80cm^3$), saturated NH_4Cl solution ($40cm^3$), H_2O ($160cm^3$), dried over $MgSO_4$ and the solvent removed *in vacuo* to leave a dark red oil. The oil was chromatographed on silica gel (DCM-->0.1% DCM/MeOH) to remove pNP and then re-chromatographed on silica gel (EtOAC/pet ether, 1:3v/v) to afford the pure product *(11)* (0.45g, 38%) after removal of solvents *in vacuo*.

υ_{max}(nujol); 3330, 3250 (NH str), 3060 (C=C str), 1750, 1680, 1650 (C=O str), 1520, 1270, 1240, 1170cm^{-1} (C-O str).

$\delta_H(CDCl_3)$; 0.93[6H, dt, $(CH_3)_2$ leu, *J*=6.0Hz], 1.45[9H, s,

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Lit [16]:- W. Konig and R. Geiger; *Chem. Ber.*, 1973,106,3626.

$(CH_3)_3$], 1.51[1H, m, CH leu], 1.68[2H, m, CH_2 leu], 2.55[2H, brm, CH_2 gly], 3.73(9), 3.74(3)[3H, sX2, OCH_3X2], 4.14[1H, brm, α_1CH leu], 4.64[1H, dq, α_2CH gly], 4.95[1H, dd, NH urethane, J=7.5, 1.5Hz], 5.13[2H, tm, $=CH_2$, J=14.0 (trans), 1.0Hz (gem)], 5,67[1H, m, -CH=], 6.69, 6.76[1H, dX2, NH amide, J=7.5 and 7.0Hz].

Found:- C,60.2; H,9.06; N,7.89. $C_{17}H_{30}N_2O_5$ requires:- C,59.7; H,8.77; N,8.19%.

Synthesis of tert-butyloxycarbonyl-L-leucine-D,L-(allyl)glycine methyl ester. (11)

$HCl.H_2N$-(allyl)Gly-OMe + Boc-Leu-OH ⟶

Boc-Leu-(allyl)Gly-OMe **(11)**

Method 3; Azide method.[Lit 17, 18] **(11)**

(7) (5.59g, 0.034M) Was warmed in DMF ($40cm^3$) to effect dissolution, and then cooled to R.T. when TEA ($94.7cm^3$, 0.034M) was added and the mixture cooled to 0^0C. **(1)** (9.92g, 0.046M, 1.4eq) was dissolved in DMF ($40cm^3$) and cooled to 0^0C, then TEA ($6.4cm^3$, 0.046M) and DPPA ($9.9cm^3$, 0.046M) added. After ca 5min the free amino component was added and the stirred reaction mixture allowed to warm slowly to R.T.

Lit [17]:- T. Shioira and S.I. Yamada; *Chem. Pharm. Bull.*,1974,22,849.

Lit [18]:- T. Shioira and S.I. Yamada; *Chem. Pharm. Bull.*;1974,22,855.

over 24h. DMF was removed in vacuo to leave an oil. The oil was dissolved in EtOAc (300cm^3) and washed with H_2O (300cm^3), citric acid (1N, 400cm^3), Na_2CO_3 (1N, 400cm^3), saturated NH_4Cl solution (400cm^3), H_2O (400cm^3), dried over $MgSO_4$ and the solvent removed in vacuo to leave an orange oil. Chromatography on silica gel (EtOAc/pet ether, 1:3v/V yielded the product (*11*) as a white solid (7.08g, 61%).

υ_{max}(nujol); 3320, 3240 (NH str), 3030 (C=C str), 1740, 1670 (C=O str), 1640, 1520, 1260, 1240cm^{-1} (C-O str).
$\delta_H(CDCl_3)$; 0.94[6H, dt, $(CH_3)_2$ leu, J=6.0 2.0Hz], 1.44[9H, s, $(CH_3)_3$], 1.52[[1H, m, CH leu], 1.68[2H, m, CH_2 leu], 2.54[2H, m, CH_2 gly], 3.74[3H, s, OCH_3], 4.16[1H, brm, α_1CH leu], 4.66[1H, m, α_2CH gly], 5.08[1H, br, NH urethane], 5.11[2H, tm, $=CH_2$, J=15.0 (trans), 5.0 (cis), 1.5Hz (gem)], 5.68[1H, m, -CH=], 6.77, 6.86[1H, dX2, NH amide, J=7.5 and 6.5Hz].
$\delta_C(CDCl_3)$; 22.1, 23.0[$(CH_3)_2$ leu], 24.8[CH leu], 28.4[$(CH_3)_3$], 36.4[CH_2 gly], 41.3[CH_2 leu], 51.6[α_1CH leu], 52.3[OCH_3], 53.1[α_2CH gly], 80.2[-CO-], 119.2[$=CH_2$], 132.2[-CH=], 155.7, 172.0, 172.5[C=O].
m/e (C.I.); 343[$(M+1)^+$, 21%], 287[$(M+1)^+-(CH_3)_3C$, 100], 243[287-CO_2, 84], 186[26], 130[61], 86[130-CO_2, 80].
Found:- C,59.6; H,9.11; N,8.19. $C_{17}H_{30}N_2O_5$ requires:- C,59.7; H,8.77; N,8.19%.

Synthesis of cyclo-L-leucine-D,L-(allyl)glycine.[Lit 6] *(13) and (14)*

Boc-Leu-(allyl)Gly-OMe ⟶

cyclo-Leu-(allyl)Gly (13),(14)

(11) (7.08g, 0.021M) Was dissolved in TFA/anisole ($60cm^3$, 5%) and stirred for 1h at R.T. while protected by a a $CaCl_2$ drying tube. The solvent was removed in vacuo and the de-protected dipeptide was used without purification or characterisation as the TFA salt, TFA.H_2N-Leu-(allyl)-Gly-OMe, which was dissolved in toluene/butanol($500cm^3$, 2:1v/v) and heated under reflux for 36h while protected by a $CaCl_2$ drying tube. The solvents were removed in vacuo to leave an off white solid, which was chromatographed on silica gel (DCM/MeOH, 25:1v/v) in order to separate the two diastereomers (13), (14). Yields were 1.95g for the anti (13) and 1.83g for the syn (14) isomers (total yield 3.78g, 86%). Both isomers were individually purified by re-crystallisation from MeOH/H_2O.

Analytical data for the anti-isomer (13):-

m.p. 238-240°C.

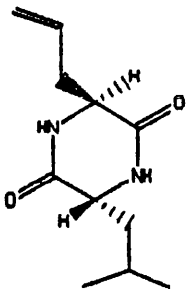
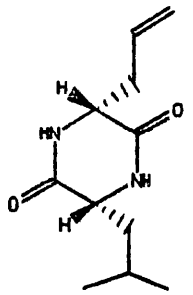
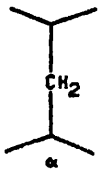
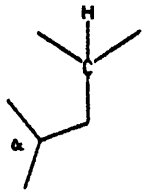
$[\alpha]_D$= +13 (c=0.28, MeOH).

υ_{max}(nujol); 3120, 3040 (NH str), 1650cm^{-1} (C=O str).

^1H and ^{13}C N.M.R.; see Tables 1 and 2 respectively.

m/e (E.I.); 210[$(M)^+$, 37%], 169[M^+-C_3H_5, 57], 154[M^+-C_4H_9, 44], 141[169-CO_2, 100], 113[154-C_3H_5, 33], 85[113-CO_2, 33].

Table 11.1. Proton chemical shift [δ_H(DMSO)] and coupling constants (Hz) of the diastereomers (*13*) and (*14*).

Proton/s	 anti (*13*)	 syn (*14*)
 Leu	0.87, q, 7.0Hz	0.87, t, 6.5Hz
 Leu	1.54, t, 7.0Hz	1.54, dm
 Leu	1.79, h, 7.0Hz	1.82, h, 6.0, 1.5Hz
αCH Leu	3.70, dt, 3.0Hz	3.76, brm

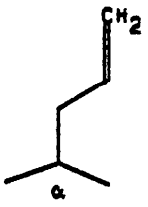
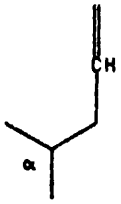
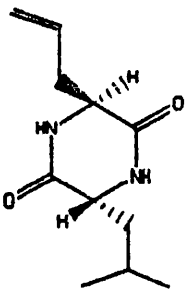
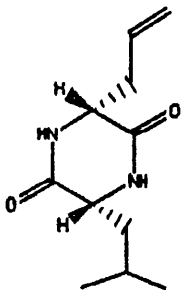
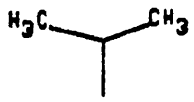
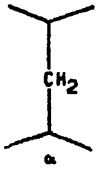
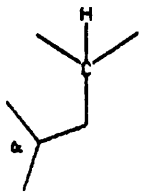
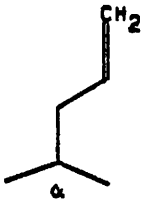
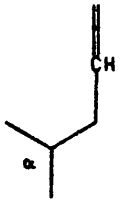
αCH Gly	3.97, t, 5.0Hz	3.91, brm
 Gly	5.12, ddt, 13.0 (trans), 9.0 (cis), 2,5Hz (gem)	5.14, ddt, 10.0 (trans), 7.0 (cis), 2.5Hz (gem)
 Gly	5.72, m	5.78, m
NH Gly	8.04, s	8.09, d, 1.5Hz
NH Leu	8.24, s	8.24, d, 1.5Hz

Table 11.2. Carbon chemical shift [δ_C(DMSO)] and coupling constants (Hz) of the diastereomers (*13*) and (*14*).

Carbon/s	 anti (*13*)	 syn (*14*)
 Leu	21.8, 22.8	21.7, 23.0
 Leu	23.4	23.4
 Leu	35.9	37.4
αCH Leu	52.8	52.4

αCH Gly	53.8	53.8
 Gly	118.9	118.6
 Gly	133.0	133.2
C=O Gly	167.3	167.0
C=O Leu	168.6	168.1

Calculated for;- $C_{11}H_{18}N_2O_2$, 210.2754; Found:- 210.1367. Found:- C,65.2; H,9.35; N,11.7. $C_{11}H_{18}N_2O_2$ requires:- C,65.6; H,9.24; N,11.8%.

A crystal of suitable dimensions was obtained from MeOH for X-ray crystallographic analysis. Details of the structure have been described in Chapter 8, section 8.6, and crystallographic data is to be found in Appendix 1.

Analytical data for the *syn*-isomer (*14*):-
m.p. 219-222°C.
$[\alpha]_D$= -53.9 (c=0.32, MeOH).
υ_{max}(nujol); 3140, 3060 (NH str), 1660cm^{-1} (C=O str).
^1H and ^{13}C N.M.R.; see Tables 1 and 2 respectively.
m/e (E.I.); 210[$(M)^+$, 43%], 169[M^+-C_3H_5, 84], 154[M^+-C_4H_9, 74], 141[169-CO_2, 100], 113[154-C_3H_5, 54], 86[113-CO_2, 54].
Calculated for:- $C_{11}H_{18}N_2O_2$, 210.2754; Found:- 210.1367.
Found:- C,65.3; H,9.61; N,11.8. $C_{11}H_{18}N_2O_2$ requires:- C,65.6; H,9.24; N,11.8%.

Synthesis of D,L-(pentenyl)-glycine methyl ester.[lit 13] *(15)*

$$PhCH{=}NCH_2CO_2CH_3 \longrightarrow HCl.H_2N\text{-(pentenyl)Gly-OMe (15)}$$

HCl.H_2N-(pentenyl)Gly-OMe (15) Was synthesised in a similar manner to that used for (7) using 5-bromo-pent-1-ene and was isolated in 63% yield.

$\delta_H(D_2O)$; 1.52[2H, brm, γ CH_2], 1.99[2H, brm, βCH_2], 2.12[2H, q, δCH_2, J=7.0Hz], 3.84[3H, s, OCH_3], 4.16[1H, t, αCH, J=6.5Hz], 5.05[2H, tm, $=CH_2$, J=15.0 (trans), 10.0 (cis), ca 1.5Hz (gem)], 5.86[1H, m, -CH=].

υ_{max}(liquid film); (benzylidine derivative); 3040, 3010 (Ar-H, HC=C str), 2840, 2910 (CH str), 1740cm^{-1} (C=O str).

m/e (C.I.); 158[$(M+1)^+$, 100%], 98[$(M+1)^+-CO_2CH_3$, 61].

Synthesis of tert-butyloxycarbonyl-D,L-(pentenyl)glycine methyl ester.[Lit 1] *(16)*

$HCl.H_2N$-(pentenyl)Gly-OMe ⟶

Boc-(pentenyl)Gly-OMe *(16)*

(15) (2.22g, 0.015M) Was dissolved in dioxane/H_2O (80cm^3, 1:1v/v), cooled to 0°C, when Na_2CO_3 (2n, 60cm^3) was added, followed by $(Boc)_2O$ (4.9g, 0.023M, 1.5eq). The reaction mixture was stirred overnight at R.T. Dioxane was removed *in vacuo* and H_2O was added to a total volume of ca 100cm^3. The aqueous layer was extracted with EtOAc (200cm^3). The extract was dried over $MgSO_4$ and the solvent removed *in vacuo* to afford the crude product which was finally chromatographed on silica gel (EtOAc/pet ether, 1:20v/v) to afford the product *(16)* as a clear oil (1.21g, 31%).

υ_{max}(liquid film); 3640 (NH str), 3060 (C=C str), 2940 (C-H str), 1700 (C=O str), 1490, 1430 (C-H def), 1160cm^{-1} (C-O str).

δ_H($CDCl_3$); 1.44[9H, s, $(CH_3)_3$], 1.62[2H, m, δCH_2], 1.81[2H, m, γ CH_2], 2.06[2H, dq, βCH_2, J=7.5Hz], 3.73[3H, s, OCH_3], 4.31[1H, q, αCH, J=7.5Hz], 5.02[2H, tm, $=CH_2$, J=18.0 (*trans*), 9.5 (*cis*), 1.5Hz (*gem*)], 5.16[1H, d, NH urethane, J=9.0Hz], 5.78[1H, m, -CH=].

δ_C($CDCL_3$); 24.42[δCH_2], 28.15[$(CH_3)_3$], 31.95[γ CH_2], 32.21[βCH_2], 51.99[OCH_3], 53.19[αCH], 79.56 [-CO-], 114.88[$=CH_2$], 137.75[$CH_2=$], 155.23[C=O urethane], 173.20[C=O ester].

m/e (C.I.); 258[$(M+1)^+$, 1.5%], 242[$(M+1)^+-CH_3$, 5.9], 202[86], 198[$(M+1)^+-CO_3CH_3$, 25], 158[$(M+1)^+-(CH_3)_3COCO$, 100], 142[49], 98[66], 81[26].

Found:- C,60.7; H,8.95; N,5.45. $C_{13}H_{23}NO_4$ requires:- C,60.7; H,9.16; N,5.43%.

<u>*Synthesis of tert-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester.*</u>[Lit 18,19] <u>*(17)*</u>

HCl.H2N-(pentenyl)Gly-OMe + Boc-Leu-OH ⟶

Boc-Leu-(pentenyl)Gly-OMe (*17*)

Boc-Leu-(pentenyl)Gly-OMe (*17*) Was prepared from (*1*) and (*15*) by a similar method to that used for the di-peptide (*11*) (<u>Method 3, Azide method</u>). The product (*17*) was isolated in 53% yield in a semi-crystalline state.

υ_{max}(nujol); 3340 (NH str), 1750, 1690, 1650 (C=O), 1530,

1170cm^{-1} (C-O).

δ_H($CDCL_3$); 0.95[6H, m, $(CH_3)_2$ leu], 1.44[9H, sX2, $(CH_3)_3$)], 1.39-1.51[4H, brm, CH_2 leu, γ CH_2 gly], 1.68[2H, m, βCH_2 gly], 1.85[1H, brm, CH leu], 2.02[2H, q, δCH_2 gly, J=7.5Hz], 3.73, 3.74[3H, sX2, OCH_3X2], 4,10[1H, brm, αCH leu], 4.58[1H, brdd, αCH gly, J=8.0Hz], 5.00[2H, tm, $=CH_2$, J=1.50 (trans), 9.5 (cis), 1.5Hz (gem)], 5.69[1H. brs, NH urethane], 5.75[1H, brm, -CH=], 6.74, 6.88[1H, brdX2, NH amide, J=7.5 and 6.5Hz].

δ_C($CDCL_3$); 22.8, 22.99[$(CH_3)_2$ leu], 24.39[γ CH_2 gly], 24.62[CH leu], 28.22[$(CH_3)_3$], 31.66, 31.75[βCH_2 gly], 33.05[δCH_2 gly], 41.00[CH_2 leu], 51.90[αCH leu], 52.22[OCH_3], 52.97[αCH gly], 115.08[$=CH_2$], 137.72[-CH=], 172.30, 172.59, 172.68[C=O amide].

m/e (C.I.); 371[$(M+1)^+$, 54%], 315[$(M+1)^+$-CH_3C, 87], 271[$(M+1)^+$-$(CH_3)_3COCO$, 66], 186[$(CH_3)_3COCONHC_5H_{10}$, 33], 130[186-$(CH_3)_3C$, 68], 86[130-CO_2, 100].

Found:- C,61.8; H,9.58; N,7.48. $C_{19}H_{34}N_2O_5$ requires:- C,61.6; H,9.19; N,7.57%.

Synthesis of cyclo-L-leucine-D,L-(pentenyl)glycine. [Lit 6] ***(18) and (19)***

Boc-Leu-(pentenyl)Gly-OMe ⟶

cyclo-Leu(pentenyl)-Gly ***(18),(19)***

Cyclo-Leu(pentenyl)-Gly ***(18),(19)*** Was prepared from H2N-Leu-(pentenyl)-Gly-OMe (from treatment of ***(16)*** with

TFA/anisole) by a similar method to that used for the preparation of the cyclic peptides (*13*) and (*14*). Chromatography was performed on silica gel (DCM/MeOH, 25:1v/v) to separate the two diastereomers. Beginning with 4.36g, 0.012M of compound (*16*), yields were 1.20g for the anti-isomer and 1.09g for the *syn*-isomer (total yield 2.29, 82%). The two diastereomers were individually purified by re-crystallisation from ($MeOH/H_2O$).

Analytical data for the anti-isomer:-

m.p. 156°C (sub), 241-242°C (dec).

$[\alpha]_D$= +6.83 (c=1.01, MeOH).

υ_{max}(nujol); 3180, 3040 (NH str), 1670cm^{-1} (C=O str).

$\delta_H(CDCL_3)$; 0.97[6H, brt, $(CH_3)_2$ leu, *J*=6.0Hz], 1.56[4H, m, γCH_2 gly, CH_2 leu], 1.80[3H, m, βCH_2 gly, CH leu], 2.10[2H, brq, δCH_2 gly, *J*=6.5Hz], 3.95[2H, brs, αCH gly, αCH leu], 4.99[2H, brt, $=CH_2-$, *J*=17.0 (trans), 10.0Hz (*cis*)], 5.77[1H, m, -CH=], 8.05[1H, brs, NH leu], 8.15[1H, brs, NH gly].

$\delta_C(CDCL_3)$; 21.18, 23.28[$(CH_3)_3$ leu], 24.10[CH leu], 24.13[γCH_2 gly], 33.15[βCH_2 gly], 33.93[δCH_2 gly], 43.83[CH_2 leu], 53.23[αCH leu], 54.91[αCH gly], 115.14[$=CH_2$], 137.72[-CH=], 168.95, 169.60[C=O].

m/e (E.I.); 238[$(M)^+$, 90%], 182[$(M)^+-C_4H_9$, 93], 170[$(M)^+-C_5H_9$, 74], 152[89], 126[NH-C_6H_{11}-CO, 31], 113[NH-C_5H_{10}-CO, 39], 98[126-CO, 72], 86[113-CO, 100]; Calculated for:- $C_{13}H_{22}N_2O_2$, 238.1860; found:- 238.1680.

Found:- C,65.2; H,9.35; N,11.7. $C_{13}H_{22}N_2O_2$ requires:- C,65.6; H,9.24; N,11.7%.

Analytical data for the *syn*-isomer (19):-

m.p. 207°C (sub), 254-256°C (dec).

$[\alpha]_D$= -35 (c=1.02, MeOH).

υ_{max}(nujol); 3180, 3050 (NH str), 1660cm^{-1} (C=O str).

m/e (E.I.); 238{$(M)^+$, 64%], 182[$(M)^+-C_4H_9$, 74], 170[$(M)^+-C_5H_9$, 66], 126[NH-C_6H_{11}-CO, 75], 113[NH-C_5H_{10}-CO, 31], 98[126-CO, 64], 86[113-CO, 90]; Calculated for $C_{13}H_{22}N_2O_2$, 238.1860; found 238.1675.

Found:- C,65.3; H,9.61; N,11.8. $C_{13}H_{22}N_2O_2$ requires:- C,65.6; H,9.24; N,11.7%.

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucine.[Lit 14] *(20)*

Boc-Leu_2-OMe ⟶ Boc-Leu_2-OH *(20)*

(3) (1.64g, 4.6mM) Was dissolved in MeOH (45cm^3) and cooled to 0°C and sufficient aqueous NaOH (1N) was added to produce a thick white precipitate and just turn the solution permanently blue in the presence of thymolphthalein indicator (*ca* pH10.5). EtOAc was added as a diluent on occasions when if the thick precipitate rendered the stirrer inoperative during the addition . MeOH and EtOAc were removed finally *in vacuo* and H_2O (50cm^3) added. The aqueous layer was extracted with EtOAc (150cm^3) and the organic layer was repeatedly washed with H_2O until it became clear. The organic layer was then dried over $MgSO_4$ and the solvent removed *in vacuo*. The product **(20)** was

recrystallised from H_2O (0.25g, 16%).

υ_{max}(nujol); 3330, 3240 (NH str), 3040 (OH str), 1750, 1670, 1640 (C=O str), 1550, 1510 (C-H def), 1150cm^{-1} (C-O str).
$\delta_H(CDCL_3)$; 0.92[12H, t, $(CH_3)_3X2$, J=6.0, 4.5Hz], 1.43[9H, s, $(CH_3)_3$], 1.47[2H, m, CH], 1.65[4H, m, CH_2], 4.22[1H, q, α_1CH, J=6.0Hz], 4.60[1H, m, α_2CH], 5.33[1H, d, NH urethane, J=8.0Hz], 7.03[1H, d, NH amide, J=8.0Hz], 8.07[1H, brs, OH, not observable on shaking with D_2O].
$\delta_C(CDCl_3)$; 21.83, 22.83[$(CH_3)_2$], 24.68[CH], 28.28[$(CH_3)_3$], 40.97, 41.45[CH_2X2], 52.22, 52.93[α_1, α_2CH], 79.95[-CO-], 155.75[C=O urethane], 172.46, 173.17[C=O].
Found:- C,59.8; H,9.30; N,7.45. $C_{17}H_{32}N_2O_5$ requires:- C,59.3; H,9.30; N,8.14%.

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucine hydrazide. [Lit 1] ***(21)***

$$Boc\text{-}Leu_2\text{-}OMe \longrightarrow Boc\text{-}Leu_2\text{-}NHNH_2 \ (21)$$

(3) (10.17g, 0.028M) Was weighed into a flamed dried flask, blanketed with N_2, and dissolved in MeOH (40cm^3). Hydrazine hydrate (4.0cm^3, 0.084M) was added and the reaction mixture stirred at R.T. for 24h. The solvents were removed *in vacuo* and the crude product chromatographed on a short silica gel column (DCM/MeOH, 20:1v/v) to afford the

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Lit [19]:- G. Guttmann and R.A. Boissonnas; *Helv. Chim. Acta.*, 1960,43,200

product (21) as a crystalline solid (10.01g, 98%).

υ_{max}(nujol); 3260 (NH str), 1700, 1630 (C=O str), 1150cm^{-1} (C-O str).

δ_H($CDCl_3$); 0.91[9H, m, $(CH_3)_2X2$], 1.44[9H, s, $(CH_3)_3$], 1.56[6H, brm, CH_2X2, CHX2], 3.95[2H, d, NH_2, J=3.5Hz], 4.18[1H, brq, α_1CH, J=6.0Hz], 4.56[1H, m, α_2CH], 5.35[1H, brd, NH urethane, J=8.0Hz], 7.19[1H, brd, NH amide, J=8.0Hz], 8.70[1H, brs, NH].

m/e (C.I.); 359[$(M+1)^+$, 12%], 303[$(M+1)^+-(CH_3)_3$, 74], 285[$(M+1)^+-(CH_3)_3CO$, 67], 271[44], 259[285-CO, 6], 225[33], 157[16], 130[14], 86[100].

Found:- C,56.57; H,9.76; N,15.5. $C_{17}H_{34}N_4O_4$ requires:- C,56.98; H,9.50; N,15.6%.

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucyl-L-leucine methyl ester. (Azide method).[Lit 2] *(5)*

$$Boc\text{-}Leu_2\text{-}NHNH_2 + H_2N\text{-}Leu_2\text{-}OMe \longrightarrow Boc\text{-}Leu_4\text{-}OMe \quad (5)$$

H_2N-Leu_2-OMe (48.8mg, 0.19mM), From treatment of (3) with TFA, was dissolved in DMF (0.3cm^3), cooled to -20^{0}C and NMM (0.021cm^3, 0.19mM) was added. (21) (61.2mg, 0.17mM) was dissolved in DMF (0.3cm^3), cooled to -20^{0}C and then treated with a 2M HCl solution in THF (0.26cm^3). The solution was stirred for ca 15min when butyl nitrite (0.004cm^3, 0.34mM)

Lit [20]:- J. Honzl and J. Rudinger; Collin. Czech. Chem. Comm., 1961,26,233.

was added, followed by NMM (0.048cm^3, 0.34mM) ca 5min afterwards. This was followed by the addition of the free amino component ca 1min afterwards. The reaction mixture was stirred at -20°C for 1h and then kept at a temperature of 4-10°C for 24h. The solvent volume was reduced by ca 30cm^3 and H_2O added to triturate the product which was finally recrystallised from EtOAc/pet ether (40.0mg, 40%).

υ_{max}(nujol); 3340, 3200, 3060 (NH str), 1670cm^{-1} (C=O str).
δ_H($CDCL_3$); 0.89[24H, m, $(CH_3)_2X4$], 1.43[9H, s, $(CH_3)_3$], 1.55[4H, m, CHX4], 1.63[8H, m, CH_2X4], 3.71[3H, s, OCH_3], 4.29[1H, brm, α_1CH], 4.63[3H, m, α_2, α_3, α_4CH] 5.58[1H, brd, NH urethane, J=8.0, 8.5Hz], 5.58[1H, brm, NH amide].
m/e (C.I.); 585[$(M+1)^+$, 25%], 511[$(M+1)^+-(CH_3)_3CO$, 52], 485[$(M+1)^+-(CH_3)_3COCO$, 53], 327[21], 86[100].
Found:- C,61.4; H,9.75; N,9.57. $C_{30}H_{56}N_4O_7$ requires:- C,61.6; H,9.59; N,9.59%.

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucyl-D,L-(pentenyl)glycine methyl ester.[Lit 20] *(22)*

$Boc\text{-}Leu_2\text{-}NHNH_2$ + H_2N-Leu-(pentenyl)Gly-OMe ⟶

$Boc\text{-}Leu_3$-(pentenyl)Gly-OMe (22)

(16) (6.46g, 0.018M) Was dissolved in TFA/anisole (45cm^3, 5%v/v) and stirred for 1h at R.T. while protected by a $CaCl_2$ drying tube. The solvent was removed in vacuo and

the deprotected dipeptide was used without purification or characterisation. *(21)* (9.03g, 0.025M, 1.4eq) was dissolved in a 2M HCl solution in THF ($130cm^3$) and cooled to $-20cm^3$ when butyl nitrite ($30cm^3$, 0.25M) was slowly added dropwise. The reaction mixture was stirred for a further 4-5min when a pre-cooled (-20^0C) solution of the free amino component in DMF ($65cm^3$) was added, followed by the dropwise addition of NMM ($28cm^3$, 0.25M). The reaction mixture was stirred and allowed to warm to overnight R.T. The solvent was removed *in vacuo* and H_2O ($600cm^3$) was added. The aqueous layer was extracted with EtOAc ($600cm^3$) and the organic layer was dried over $MgSO_4$. The solvent was removed *in vacuo* and the crude product was chromatographed on silica gel (EtOAc/pet ether 1:3v/v) to afford the product *(22)* as a semi-crystalline solid (5.37g, 52%).

υ_{max}(nujol); 3320, 3280 (NH str), 1750, 1720, 1690, 1640 (C=O str), 1530, 1170cm^{-1} (C-O str).

δ_H(DMSO); 0.84[18H, $(CH_3)_2$X3 leu], 1.37[9H, s, $(CH_3)_3$], 1.40[6H, m, CH_2X3, γ CH_2 gly], 1.56[5H, brm, CHX3, βCH_2 gly], 2.00[2H, quin, δCH_2, J=6.5Hz], 3.59, 3.60[3H, s, OCH_3X2], 3.91[1H, brq, α_1CH leu, J=8.5Hz], 4.23[1H, brm, α_1CH gly], 4.35[2H, br quin, α_2, α_3CH leu, J=7.0, 1.5Hz], 4.97[2H, tm, $=CH_2$, J=14.0 (trans), 9.0 (cis), 2.0Hz (gem)], 5.76[1H, brm, -CH=], 6.94[1H, d, NH urethane, J=7.5Hz], 7.85[2H, brm, NH amideX2], 8.22, 8.25[1H, dX2, NH glyX2, J=7.5 and 7.5Hz].

δ_C(DMSO); 21.55, 21.65, 22.84, 22.90, 22.97[$(CH_3)_2$ leu, two

co-incident peaks], 23.91, 24.00, 24.13 [CH leu], 24.26, 24.36[γ CH_2 gly], 32.44, 32.57[βCH_2 gly], 40.48, 40.67, 40.84[CH_2 leu], 50.47, 50.60, 50.76[$\alpha_1, \alpha_2, \alpha_3$CH leu], 51.44, 51.57[$\alpha$CH gly], 52.81[$OCH_3$], 77.91[-C-O-C=O], 114.92[$=CH_2$], 138.11[-CH=], 155.20[C=O urethane], 171.36, 171.42, 171.91, 172.26, 172.33[C=O amide].

m/e (C.I.); 597[$(M+1)^+$, 3%], 541[$(M+1)^+-(CH_3)_3C$, 5], 497[$(M+1)^+(CH_3)_3COCO$, 2], 454[$(M+1)^+-C_6H_{10}CO_2CH_3$, 2], 440[454-NH, 1], 358[36], 130[13], 86[100].

Found:- C,61.8; H,9.41; N,9.25. $C_{31}H_{56}N_2O_7$ requires:- C,62.4; H,9.40; N,9.40%.

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucyl-D,L-(pentenyl)glycine hydrazide. [Lit 20] ***(23)***

$Boc\text{-}L\text{-}Leu_3\text{-}(pentenyl)Gly\text{-}OMe \longrightarrow$

$Boc\text{-}Leu_3\text{-}(pentenyl)Gly\text{-}NHNH_2$ (23)

(22) (0.5945g, 0.9975mM) Was weighed into a flask and quickly flame-dried under a stream of N_2 gas. The solid was dissolved in MeOH ($40cm^3$) and hydrazine hydrate ($0.15cm^3$, 3eq) was added. The reaction mixture was stirred at R.T. for 48h in a sealed flask. The solvent was removed in vacuo and the crude product chromatographed on silica gel (DCM/MeOH, 30:1v/v) to afford the product (23) as a white solid (0.5129g, 86%, m.p. 219-220°C.).

δ_H(DMSO); 0.85[18H, m, $(CH_3)_2$X3 leu], 1.37[9H, s, $(CH_3)_3$], 1.42[6H, m, CH_2X3 leu, γ CH_2 gly], 1.56[5H, brm, CHX3, βCH_2 gly], 1.97[2H, quin, δCH_2, gly, J=6.5Hz], 3.92[1H, brq, α_1CH leu, J=8.5Hz], 4.21[2H, m, NH_2], 4.33[3H, m, α_2, α_3CH leu, α_4CH gly], 4.95[2H, td, $=CH_2$, J=16.0 (trans), 9.5 (cis), 2.5Hz (gem)], 5.77[1H, m, -CH=], 6.94[1H, d, NH urethane, J=10.0Hz], 7.8, 8.0[3H, m and brt, NH amides, J=8.0Hz], 9.10[1H, brs, NH].

δ_C(DMSO); 21.34, 21.47, 21.54, 21.60, 22.87, 22.96[$(CH_3)_2$ leu], 23.91, 24.00, 24.13[CH leu], 24.33[δCH_2 gly], 28.06[$(CH_3)_3$], 31.62, 32.88[γ CH_2 gly], 32.57, 32.73[βCH_2 gly], 40.51, 40.67, 40.84[CH_2 leu], 50.57, 50.73, 51.18[α_1, α_2, α_3CH leu], 52.74[α_4CH gly], 77.91[-CO- urethane], 114.79[$=CH_2$], 138.24[-CH=], 155.20[-C=O urethane], 170.58, 171.29, 171.52, 171.61, 172.30[C=O amide].

m/e (C.I.); 597[$(M+1)^+$, 41%], 86[100].

Found:- C,60.6; H,9.61; N,13.9. $C_{30}H_{56}N_6O_6$ requires:- C,60.4; H,9.61; N,14.1%.

<u>*Attempted synthesis of cyclo-L-leucyl-L-leucyl-L-leucyl-D,L-(pentenyl)glycine.*[Lit 3] *(24)*</u>

Boc-Leu_3-(pentenyl)Gly-OMe ⟶ cyclo-Leu_3-(pentenyl)Gly (24)

Lit [21]:- R. Schwyzer and P. Sieber; *Helv. Chim. Acta.*, 1957,40,624.

Method 1.

(22) (0.9788g, 1.64mM) Was dissolved in TFA/anisole ($4cm^3$ v/v) and stirred for 1h at R.T. while protected by a $CaCl_2$ drying tube. The solvent was removed *in vacuo* and the de-protected tetra-peptide was used without purification of characterisation as the TFA salt, TFA.H_2N-Leu_3-(pentenyl)Gly-OMe, which was dissolved in DMF ($16cm^3$) and 5 drops of glacial acetic acid added. The solution was slowly added dropwise to pyridine ($60cm^3$) heated to ca 65^0C. The reaction mixture was allowed to stir at 65^0C for 3 days. The solvents were removed *in vacuo* to leave a yellow solid which was chromatographed on silica gel (DCM/MeOH, 25:1v/v), to give the major product (0.5694g, 70%) which was not the cyclic peptide (24), but the linear tetra-peptide methyl ester which was unprotected at the amino end.

υ_{max}(nujol); 3250, 3040 (NH str), 1750, 1690, 1650, 1630 (C=O str), $1540cm^{-1}$.

δ_H(DMSO); 0.85[18H, q, $(CH_3)_2$X3 leu, J=6.5Hz], 1.44[6H, m, CH_2X3 leu, γ CH_2 gly], 1.58[5H, brm, CHX3 leu, βCH_2 gly], 2.00[2H, br quin, δCH_2 gly, 5.5Hz], 3.61, 3.62[3H, sX2, OCH_3], 4.25[1H, brm, α_1CH gly], 4.34[3H, brm, $\alpha_1, \alpha_2, \alpha_3$CH leu], 4.97[2H, tm, $=CH_2$, J=17.0 (trans), 8.5 (cis), 1.0Hz (gem)], 5.77[1H, brm, -CH=], 7.84[2H, dX2, NH gly, J=8.4Hz], 8.14, 8.19, 8.24[3H, dX3, NH amide, J=7.0, 6.5 and 8.0Hz respectively].

m/e (C.I.); 496[$(M+1)^+$-H, 15%], 368[15], 149[49], 86[100].

Found:- C,62.6; H,9.46; N,10.2. $C_{26}H_{48}N_4O_4$ requires:- C,62.9, H,9.68; N,11.3%.

Method 2.

(23) (0.5552g, 0.9315mM) Was dissolved in TFA/anisole ($8cm^3$, 5%v/v) and stirred for 1h at R.T. under a $CaCl_2$ drying tube. The solvent was removed *in vacuo* and the de-protected tetra-peptide was used without purification of characterisation as the TFA salt. TFA.H_2N-Leu_3-(pentenyl)Gly-OMe was dissolved in DMF ($3.2cm^3$), treated with a 2M HCl solution in THF ($4.7cm^3$) and cooled to -20°C when butyl nitrite ($1.1cm^3$, 9.315mM) was slowly added dropwise. The reaction mixture was stirred for a further 5min and diluted with pre-cooled DMF ($23cm^3$) when NMM ($1.1cm^3$, 9.315mM) was slowly added dropwise. The stirred mixture was allowed to warm to R.T. overnight, then the solvents removed *in vacuo* and H_2O ($25cm^3$) added. The aqueous layer was extracted with EtOAc ($50cm^3$) and the organic layer dried over $MgSO_4$. T.L.C. analysis of the crude product indicated the formation of a multi-component mixture which was not chromatographed or characterised.

Synthesis of tert-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucyl-D,L-(pentenyl)glycine. [Lit 14] ***(25)***

Boc-Leu_3-(pentenyl)Gly-OMe ⟶

Boc-Leu_3-(pentenyl)Gly-OH (25)

(22) (0.9702g, 1.63mM) Was dissolved in MeOH (13cm^3) and cooled to 0°C when aqueous NaOH (1M, 5.0cm^3, 1.5eq) was added dropwise, to give a precipitate. The suspension was stirred at 0°C for a further 3h when a further 5.0cm^3 of NaOH (5.0cm^3) was added. The reaction mixture was stored at ca 5°C for ca 12h. The resultant clear solution was acidified with HCl (1M, 10.0cm^3) to precipitate a solid which was filtered off, washed with water, dissolved in MeOH (25cm^3), and stored over molecular sieves (type 3Å) for 24h to dry. The dissolved product was decanted, and MeOH was removed in vacuo to afford the product (25) as a white solid (0.9153g, 97%).

δ_H(DMSO); 0.84[18H, t, $(CH_3)_2$X3 leu, J=6.5Hz], 1.36[9H, s, $(CH_3)_3$], 1.43[6H, m, CH_2X3 leu, γ CH_2 gly], 1.58[5H, m, CHX3 leu, βCH_2 gly, J=6.5Hz],2.08[2H, quin, δCH_2 gly], 3.95[1H, brq, α_1CH leu, J=8.0Hz], 4.08[2H, brm, α_2, α_3CH leu], 4.31[2H, br sex, α_4CH gly, J=8.0Hz], 4.94[2H, t, $=CH_2$, J=17.0 (trans), 10.0Hz (*cis*)], 5.72[1H, brm, -CH=], 6.92[1H, dd, NH urethane, J=8.0, 3.0Hz], 7.76, 7.85, 8.03[3H, d, q and brt respectively, J=7.5, 6.5 and 9.5Hz

respectively].

δ_C(DMSO); 21.57, 21.67, 22.90, 23.03[$(CH_3)_2$X3 leu], 23.97, 24.07, 24.20[CHX3 leu], 24.16, 24.33[δCH_2 gly], 28.09[$(CH_3)_3$], 31.17, 32.50[γ CH_2 gly], 32.86, 32.92[βCH_2 gly], 40.64, 40.87, 41.13[CH_2X3 leu], 50.63, 51.25, 51.54[$\alpha_1, \alpha_2, \alpha_3$CH leu], 52.29, 52.84[$\alpha_4$CH gly], 77.91[-CO-urethane], 114.63[=CH_2], 138.08[-CH=], 155.20[-C=O urethane], 171.16, 171.36, 171.52, 171.58[C=O amide], 173.89, 173.98[C=O carboxyl].

m/e (C.I.); 583[$(M+1)^+$, 3%], 483[$(M+1)^+$-$(CH_3)_3COCO$, 5%], 327[$(M+1)^+$-$C_{17}H_{31}N_2O_4$, 20], 271[17], 227[16], 149[21], 130[14], 113[10], 98[10], 86[130-CO_2, 100].

Synthesis of phenylpropyltriethylsilane.[Lit 4] *(26)*

$$C_6H_5\text{-}CH_2\text{-}CH{=}CH_2 + HSiEt_3 \longrightarrow C_6H_5\text{-}(CH_2)_3\text{-}SiEt_3 \ (26)$$

Method 1.

Allylbenzene (1.18g, 1.3cm^3, 10mM) and triethylsilane (1.16g, 1.6cm^3, 10mM, 1.0eq) were dissolved in toluene/butan-2-ol (2cm^3, 2:v/v) and treated with H_2PtCl_6 catalyst, dissolved in toluene/butan-2-ol (ca 10^{-4} mol per mol of allyl benzene). The reaction mixture was heated under reflux for 24h. The solvents were removed in vacuo and the crude product chromatographed on silica gel (pet ether) to afford the product *(26)* as an oil (2.47g, 53%).

Lit [22]:- J.L. Spier, J.A. Webster and G.H. Barnes; J. Amer. Chem. Soc., 1974, 57, 974

υ_{max}(nujol); 3020, 3000 (Ar-H str), 2880 (C-H str), 1950-1740 (mono-substitution overtones), 1580, 1480, 1440, 1400, 1370, 1340 (aromatic C=C str, CH_2, CH_3 str), 710, 680cm^{-1} (aromatic C-H).

$\delta_H(CDCl_3)$; 0.50[6H, m, CH_3CH_2Si-], 0.90[9H, t, $C\underline{H}_3CH_2Si$, J=8.0Hz], 1.59[2H, brm, γ CH_2], 1.81[2H, dd, βCH_2, J=6.5, 1.5Hz], 2.55[2H, t, δCH_2, J=7.0Hz], 7.10-7.27[5H, m, Ar-H].

$\delta_C(CDCL_3)$; 3.34[CH_3-$\underline{C}H_2Si$-], 7.43[$\underline{C}H_3$-CH_2Si], 11.29[δCH_2], 26.11[γ CH_2], 40.25[βCH_2], 125.30, 125.59, 125.82, 126.66, 128.18, 128.41[aromatic C=C].

m/e; no M^+ or $(M+1)^+$ ion present at 234 mass units; 205[M^+-C_2H_5, 83%].

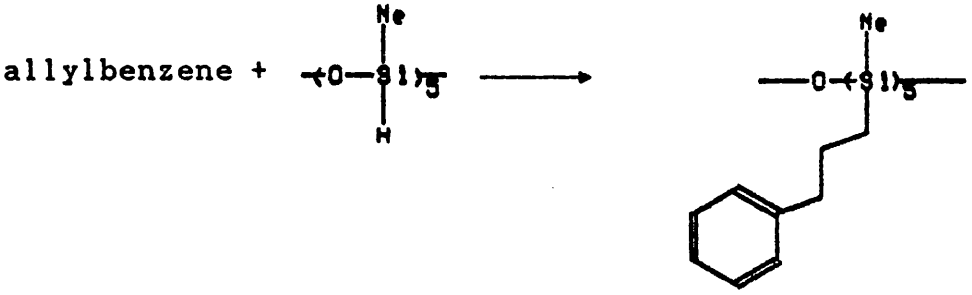
Found:- C,80.0; H,10.9. $C_{15}H_{26}Si$ requires:- C,79.9; H,11.1%.

Method 2.

Synthesis of (26) using Cp_2PtCl_2 as catalyst was performed by a similar method. Beginning with 2,36g of allylbenzene and 2.22g of triethylsilane, the yield of the desired product (26) was 2.85g (55%), and it was confirmed as being identical all respects to the product synthesised by Method 1.

Synthesis of Me_3-{$OSiMe_2$}$_3$-{$OSiMe[(CH_2)_3C_6H_5]$}$_5$-$OSiMe_3$.[Lit 22] (27)

Reaction of siloxane polymer $[Me_3Si(OSiMe_2)_3(OSiMeH)_5OSiMe_3$ with allyl benzene.



27

The siloxane polymer (3.1g, 45mM) and allyl benzene (3.45g, 0.03M) were dissolved in toluene/butan-2-ol ($1cm^3$, 2:1v/v) and treated with H_2PtCl_6 catalyst, dissolved in toluene/butan-2-ol (*ca* 10^{-4} mol per mol of siloxane polymer). The reaction mixture was heated under reflux for 48h while protected by $CaCl_2$ drying tube. The progress of the reaction was monitored by observing the Si-H peak in the I.R. spectrum (ca $2130cm^{-1}$). Solvents were removed *in vacuo* and the clear oil chromatographed on silica gel ($CHCl_3$/pet ether, 5%v/v) to afford the product **(27)** as a clear oil (5.90g, 86%).

υ_{max}(nujol); 3040 (Ar-H str), 2940, 1590 (Ar, C=C str), 1240 (Si-CH_3 asym and sym str), 1100, 1000 (Si-O-Si asym str).
$\delta_H(CDCl_3)$; 0.00[51H, brm, Me_3SiO and SiMe], 0.48[10H, brm, Si-CH_2], 1.58[10H, brm, $SiCH_2$-CH_2], 2.52[10H, brm, $SiCH_2CH_2$-

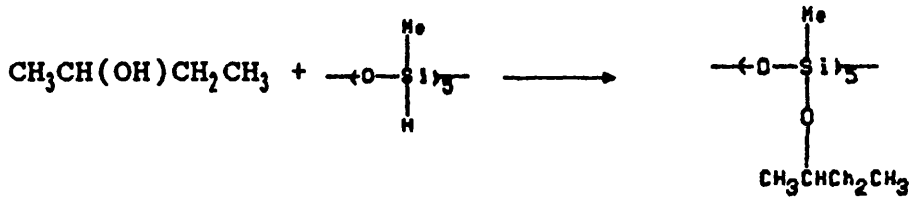
$C\underline{H}_2$], 7.12[25H, Ar-H].

$\delta_C(CDCl_3)$; -3.1[MeSiO], 1.2[Me_2SiO, 1.7[$SiMe_3$], 17.3[Si-$\underline{C}H_2$], 25.[$SiCH_2$-$\underline{C}H_2$], 39.5[$SiCH_2CH_2$-$\underline{C}H_2$], 125.6, 128.2, 128.4, 142.6[Ar-H].

m/e; no M+ or $(M+1)^+$ ion present at 1274 mass units; 177{$[OSi(CH_3)(CH_2)_3C_6H_5]^+$, 30%}, 147{$[Si(CH_3)_2OSi(CH_3)_3]^+$, 3}, 73[$(CH_3)_3SiO)^+$, 100].

Synthesis of Me_3-{$OSiMe_2$}$_3$-{$OSiMe[OCH(CH_3)CH_2CH_3]$}$_5$-$OSiMe_3$. Lit 22 *(28)*

Reaction of siloxane polymer [Me_3Si-$(OSiMe_2)_3$-$(OSiMeH)_5$-$OSiMe_3$ with butan-2-ol.



28

The siloxane polymer (1.0g, 1.5mM) was dissolved in toluene/butan-2-ol ($30cm^3$, 2:1v/v) and treated with H_2PtCl_6 catalyst, dissolved in toluene/butan-2-ol (*ca* 10^{-4} mol per mol of siloxane polymer). The reaction mixture was heated under reflux while protected by a $CaCl_2$ drying tube for 24h. The solvents were removed *in vacuo* and the oil chromatographed on silica gel ($CHCl_3$/pet ether 1:4v/v) to afford the major product *(28)* as a clear oil (0.56g, 37%).

υ_{max}(nujol); 2160 (Si-H str), 1255 (Si-CH_3 str), 1050 (Si-O-Si br aliphatic peak), 800cm^{-1} (Si-CH3).

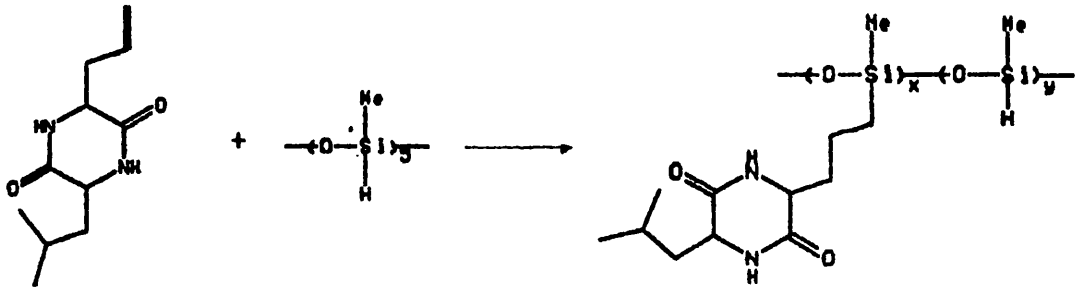
δ_H($CDCl_3$); 0.10[51H, brm, CH_3SiO, $SiCH_3$], 0.87[15H, t, $\underline{CH_3}$-CH_2, J=7.5Hz], 1.16[15H, m, CH_3-CH], 1.46[10H, brm, CH_3-$C\underline{H_2}$], 3.91[5H, brm, -CH-].

δ_C($CDCl_3$); -3.25[CH_3SiO], 0.94[$(CH_3)_2SiO$], 1.75[$(CH_3)_3SiO$, 9.96[$\underline{C}H_3$-CH_2], 22.93[$\underline{C}H_3$-CH], 32.01[CH_3-$\underline{C}H_2$], 69.64[SiO-CH].

m/e; no M+ or $(M+1)^+$ ion present at 1374 mass units; 73[$(CH_3)_3Si$]

Synthesis of Me_3-Si{$OSiMe_2$}$_3$-{OSiMe[cyclo-Leu-(allyl)Gly]}-$OSiMe_3$. [Lit 22] ***(29)***

Reaction of siloxane polymer $[Me_3Si(OSiMe_2)_3(OSiMeH)_5OSiMe_3$ with cyclo-Leu-(allyl)Gly.



x+y=5

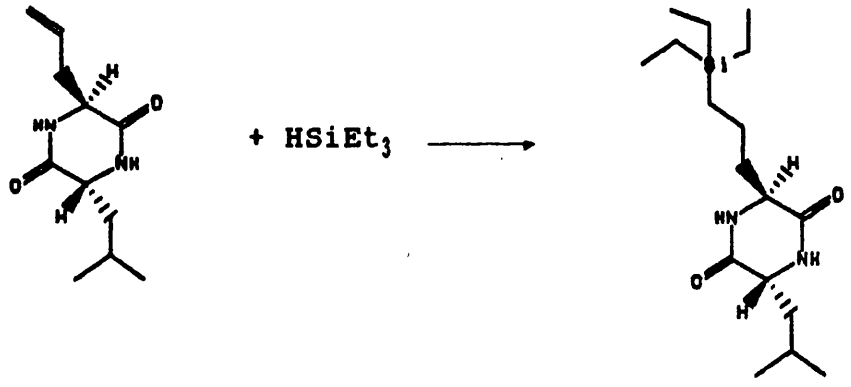
29

The cyclo-peptide (5mg, 0.02mM) and the siloxane polymer (260Mg, 0.38mM) was suspended in toluene/MeCN (60cm^3, 2:1v/v), treated with H_2PtCl_6 catalyst, dissolved in

toluene/MeCN (ca 10^{-4} mol per mol of cyclic peptide), and heated at 80-90°C for 6 days, with the addition of H_2PtCl_6 catalyst every 24h. The solvents were removed in vacuo and the residue chromatographed on silica gel ($MeOH/CHCl_3$, 2%v/v) to afford 54mg of the major product which was weakly UV active and unreactive towards $KMnO_4$ (white spot) indicator.

δ_C(DMSO); 0.9[Me_3SiO], 1.6[Me_2SiO], 1.7[MeSiO], 17.9[δCH_2 gly], 21.4, 23.1[[$(CH_3)_2$ leu], 24.1[CH leu], 29.7[βCH_2 gly], 35.3[γ CH_2 gly], 42.4[CH_2 leu], 53.3[αCH gly], 54.6[αCH leu], 169.1[C=O].

<u>*Synthesis of cyclo-L-leucine-D-(allyl)glycine triethyl silane.*</u>[Lit 22] <u>*(30)*</u>



30

(13) (0.275g, 1.18mM) Was suspended in toluene/MeCN ($8cm^3$, 4:1v/v, degassed under N_2), and under N_2, $HSiEt_3$ ($0.375cm^3$, 2.36mM, 2eq) was added. Cp_2PtCl_2 catalyst,

dissolved in toluene/MeCN (ca 10^{-4} mol per mol of cyclic peptide) was added, the reaction tube sealed, and then heated to 150°C. After 24h the tube was opened under N_2 gas and a further volume a catalyst was added. The tube was re-sealed and heated for a further 24h after which, the tube was allowed to cool to R.T., and MeOH was added to the solidified solution. The solvents were removed *in vacuo* to leave a solid which was chromatographed on silica gel (DCM/MeOH, 9:1v/v) to afford the product (*30*) (0.3344g, 87%, m.p. 241-242°C.).

$[\alpha]_D$= -8.2 (c=0.135, MeOH).

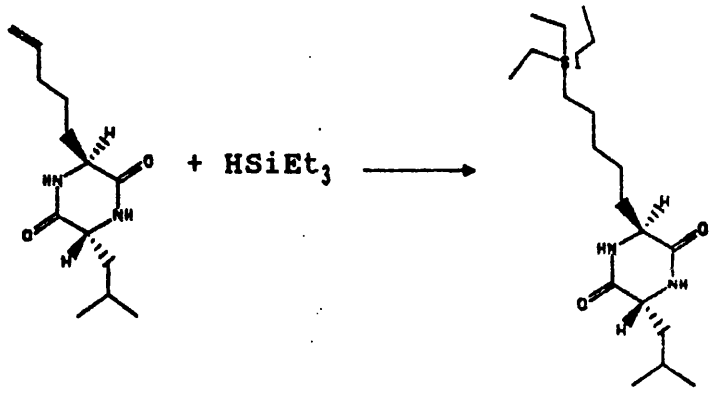
υ_{max}(nujol); 3270, 3040 (NH str), 1650 (C=O str), 1320, 1140, 1080, 1000, 820, 720cm^{-1}.

δ_H(DMSO); 0.47[8H, q, CH_3-$C\underline{H}_2$-SiX3, δCH_2 gly, J=8.0Hz], 0.89[15H, $C\underline{H}_3$-CH_2SiX3, $(CH_3)_2$ leu, J=6.5, 8.5Hz], 1.31[2H, m, γ CH_2 gly], 1.53[2H, dt, CH_2 leu, J=7.0, 1.5Hz], 1.68[2H, m, βCH_2 gly], 1.78[1H, m, CH leu], 3.68[1H, brdt, αCH leu, 6.5, 2.0Hz], 3.83[1H, brt, αCH gly, J=5.0Hz], 8.12[1H, brs, NH gly], 8.15[1H, brd, NH leu, J=2.5Hz].

δ_C(DMSO); 2.95[CH_3-$\underline{C}H_2$-Si], 7.47[$\underline{C}H_3$-CH_2Si], 10.96[δCH_2 gly], 18.49[γ CH_2 gly], 21.92, 22.93[$(CH_3)_2$ leu], 23.61[CH leu], 35.97[βCH_2 gly], 42.03[CH_2 leu], 52.90[αCH leu], 53.58[αCH gly], 168.21, 168.92[C=O],

m/e (C.I.); 327[$(M+1)^+$, 7%], 297$(M+1)^+$-CH_3CH_2, 57], 213[$(M+1)^+$-$SiEt_3$, 100], 170[19], 156[213-C_4H_9, 49].

Synthesis of cyclo-L-leucine-D-(pentenyl)glycine triethyl silane.[Lit 22] **(31)**



31

Method 1. Under nitrogen gas at atmospheric pressure.

(18) (50.8mg, 0.21mM0 Was suspended in toluene/MeCN ($2cm^3$, 3:1v/v), $HSiEt_3$ ($0.17cm^3$, 1.67mM, 5eq) and treated with Cp_2PtCl_2 catalyst, dissolved in toluene/MeCN (ca 10^{-4} mol per mol of cyclic peptide). The reaction was heated to 80°C under an atmosphere of N_2. A further volume of catalyst was added 24h later and the reaction continued for a further 32 days. The reaction mixture was allowed to cool to R.T. and MeOH was added to dissolve the solidified solution. The solvents were removed *in vacuo* and the crude product chromatographed on silica gel (EtOAc/pet ether, 1:4->EtOAc) to afford the product *(31)* as a white solid (33.7mg, 45%).

Method 2. Under nitrogen gas in a pressurised reaction tube.

(18) (0.0488g, 21mM) Was suspended in toluene/MeCN ($2cm^3$, 3:1v/v, degassed under N_2), $HSiEt_3$ ($0.16cm^3$, 1.03mM, 5eq) and treated with Cp_2PtCl_2 catalyst, dissolved in toluene/MeCN (ca 10^{-4} mol per mol of cyclic peptide). The reaction tube was sealed and heated to 150°C for 7 days, after which the tube was re-opened under N_2 and a further volume a catalyst was added. The tube was re-sealed and heated for a further 7 days. The reaction tube was allowed to cool to R.T. and MeOH was added to the solidified solution. The solvents were removed *in vacuo* to leave a solid which was chromatographed on silica gel (DCM/MeOH, 45:1v/v) to afford the product *(31)* (0.0354g, 48%).

The above experiment was repeated as described above (Method 2) except that the reaction tube was heated for 48h at 150°C with an addition a a further volume of catalyst after 24h. The yield of the product *(31)* was 31%.

m.p. 207-209°C.

$[\alpha]_D$= +1.9 (c=0.271, MeOH).

υ_{max}(nujol); 3270, 3040 (NH str), 1650 (C=O str), 1330, 1120, 1080, 1000, 960, 810, 720cm^{-1}.

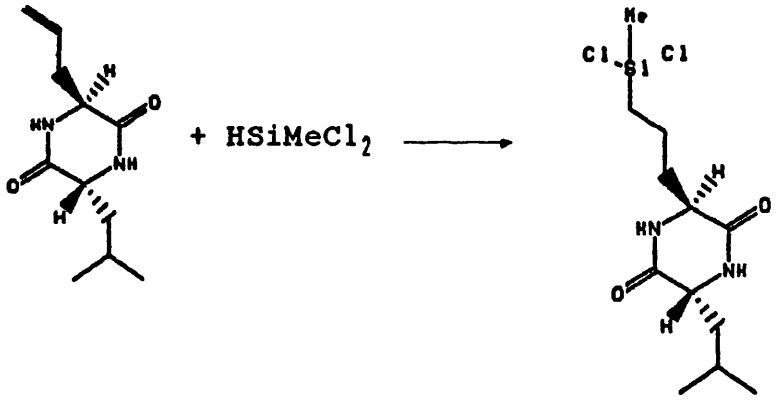
δ_H(DMSO); 0.47[8H, qd, CH_3-$C\underline{H}_2$-SiX3, ζCH_2 gly, J=8.0Hz], 0.87[15H, m, $C\underline{H}_3$-CH_2Si, $(CH_3)_2$ leu], 1.27[6H, m, γCH_2, δCH_2, ϵCH_2 gly], 1.53[2H, dt, CH_2 leu, J=7.5, 1.5Hz], 1.64[2H, brm, βCH_2 gly], 1.79[1H, dq, CH leu, J=7.0Hz], 3.70[1H, brdt, αCH leu, J=6.5, 2.5Hz], 3.83, brdd, αCH gly, J=5.0Hz], 8.08[1H,

brs, NH gly], 8.15[1H, d, NH leu, J=2.5Hz].

δ_c(DMSO); 2.92[CH_3-$\underline{C}H_2$-Si], 7.39[$\underline{C}H_3$-CH_2Si], 10.76[ζCH_2 gly], 21.96, 22.90[$(CH_3)_2$ leu], 23.29, 23.48[δCH_2, εCH_2 gly], 23.58[CH leu], 31.75[γCH_2 gly] 33.08[βCH_2 gly], 42.03[CH_2 leu], 52.87[αCH leu], 53.61[αCH gly], 168.11, 168.82[C=O].

m/e (C.I.); 355[$(M+1)^+$, 8%], 325[$(M+1)^+-C_2H_5$, 29], 279[325-C_2H_5, 22], 167[23], 149[100], 113[23], 71[20].

Synthesis of cyclo-L-leucine-D-(allyl)glycine dichloro-methyl silane.[Lit 22] *(32)*



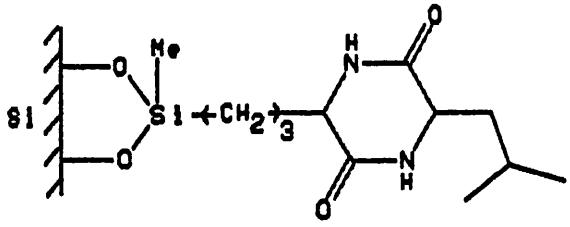
32

(13) (17.2mg, 0.082mM) Was dissolved in dry toluene/DCM (30cm^3, 2:1v/v) and treated with H_2PtCl_6 catalyst, dissolved in toluene/MeCN (ca 10^{-4} mol per mol of cyclic peptide). After *ca* 15min stirring, $HSiMeCl_2$ (0.1cm^3, 0.97mM, 12eq) was slowly added dropwise. The reaction mixture was slowly stirred at R.T. for 48h and then heated

under reflux for 30min. The solvents were removed *in vacuo* and excess $HSiMeCl_2$ was removed by azeotropic distillation with DCM. The product (32) readily hydrolyses on exposure to moist air. ^1H NMR spectroscopic data only was obtained due to handling difficulties. Subsequent syntheses of this compound were used at once without purification or characterisation.

$\delta_H(CD_2Cl_2)$; 0.00-0.04[15H, brm, Si-CH_3], 0.82[6H, brm, $(CH_3)_2$ leu], ca 1.0-2.0[9H, brm, CH_2, CH leu, βCH_2, γCH_2, δCH_2 gly], 4.23[2H, brm, αCH leu, αCH gly], 7.65[1H, m, NH leu], 7.67[1H, m, NH gly].

Preliminary investigation into the derivatisation of silica gel based supports. [Lit 5] ***(CSP 1)***



CSP 1

(13) (104.4mg, 0.50mM) Was suspended in dry DCM (50cm^3) and treated with H_2PtCl_6 catalyst, dissolved in toluene/MeCN, (ca 10^{-4} mol per mol of cyclic peptide). The

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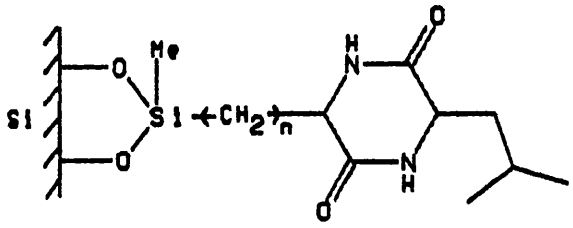
Lit [23]:- W.H. Pirkle, T.C. Pochaphsky, G.S. Mahler, D.E. Corey, D.S. Reno and M. Alessi; *J. Org. Chem.*, 1986,51,4991

solution was stirred at R.T. for *ca* 5min and then $HSiMeCl_2$ ($0.052cm^3$, 0.5mM, 1.0eq) was added dropwise. The reaction mixture was stirred at R.T. for a further 30min and heated under reflux under an atmosphere of dry N_2 for 48h. The solution was transferred to a suspension of silica gel (1.0g 12µm, 90% ± 7µm) in toluene ($10cm^3$), TEA ($7.0cm^3$) was added dropwise and the suspension stirred at R.T. for 1h and then at reflux for 3h. The solvents were removed by distillation at aspirator pressure, and the treated silica heated at 125°C for 24h *in vacuo*. The beige coloured silica was washed with MeOH, MeCN, MeCN/H_2O (1:1v/v), H_2O and MeOH to remove un-reacted cyclic peptide and the silica was finally dried at 40°C *in vacuo*. An untreated sample of silica was similarly washed for comparison.

Corrected elemental analysis for the treated silica gave:- C,9.37; H,0.67; N,0.06%; giving a loading of 0.32mmol/g and $0.30mMg^{-1}$ of silica gel based on the carbon and hydrogen analyses respectively.

Treatment of high performance liquid chromatography silica gel with cyclo-L-leucine-L-(allyl)glycine and cyclo-L-leucine-L-(pentenyl)glycine.[Lit 23] *(CSP 2) and (CSP 3)*



n=3,5

(14) (0.525g, 2.5mM) Was suspended in dry DCM ($100cm^3$) and the reaction vessel blanketed with N_2 before treating with H_2PtCl_6 catalyst, dissolved in toluene/MeCN (ca 10-4 mol per mol of cyclic peptide). The suspension was stirred for ca 15min and then HSiMeCl2 ($0.26cm^3$, 2.5mM, 1.0eq) was added. The reaction mixture was heated under reflux for 24h. A further volume of HSiMeCl2 was added and the reaction continued for a further 24h. DCM was distilled off and DCM ($100cm^3$) was added as a "chaser". The volume was reduced by ca $10cm^3$ and the solution transferred to a toluene ($150cm^3$) suspension of silica gel (5.0g), which had been oven dried for 48h at 130°C. The suspension was blanketed with N_2 and the suspension heated under reflux for 3 days. The solvents were distilled off leaving an off white powder which was oven cured at 100°C for 24h. The silica was washed three times with MeCN and then with H_2O and once with MeOH before oven drying at 65°C for 24h. An untreated sample of silica gel was similarly washed for

comparison. The treated silica gel weighed 5.55g.

Corrected elemental analysis for the treated silica gel gave:- C,4.93; H,0.66; N,0.95%; giving a loading of 0.34, 0.30 and 0.34mMg^{-1} of silica gel based on the carbon, hydrogen and nitrogen analyses respectively. An internal check for the correct C:H:N ratio gave:- 7.47:1.00:1.44. $C_{12}H_{22}N_2$ requires:- 6.55:1.00:1.27.

Treatment of silica gel (5.0g) with (19) (0.595g, 0.25mM) was achieved similarly. The silica gel weighed 5.63g after derivatisation.

Corrected elemental analysis for the treated silica gel gave:- C,4.82; H,0.62; N,0.75%; giving a loading of 0.29, 0.24, 0.27mMg^{-1} of silica gel based on the carbon, hydrogen and nitrogen analyses respectively. An internal check for the correct C:H:N ratio gave:- 7.77:1.00:1.21. $C_{14}H_{26}N_2$ requires:- C,6.46; H,1.00; N,1.08.

APPENDIX ONE

Table A.1-A.5. Supplementary data of anisotropic factors, and hydrogen poistions for cyclo-L-leucine-D-(allyl)glycine (13).

Table A.1. Fractional atomic co-ordinates and thermal parameters (Å) for cyclo-L-leucine-D-(allyl)glycine (13).

Atom	x	y	z	Uiso or Ueq (***)	
O1	0.55977	0.0590	-0.4901(6)	0.37(4)	***
O2	0.4375(7)	-0.0061(9)	-0.0009(6)	0.039(4)	***
N1	0.4670(8)	-0.1094(12)	-0.3419(7)	0.032(4)	***
N2	0.5112(8)	0.1676(11)	-0.1541(6)	0.032(4)	***
C1	0.5217(9)	0.0436(14)	-0.3805(8)	0.026(5)	***
C2	0.5425(10)	0.2017(15)	-0.2869(8)	0.030(4)	***
C3	0.4576(9)	0.0149(13)	-0.1169(8)	0.026(5)	***
C4	0.4013(9)	-0.1316(13)	-0.2216(7)	0.026(4)	***
C5	0.7238(11)	0.2908(13)	-0.2585(9)	0.042(5)	***
O6	0.8740(12)	0.1716(16)	-0.1821(11)	0.053(6)	***
C7	1.0008(13)	0.2144(18)	-0.0658(11)	0.064(7)	***
C8	0.1959(10)	-0.1416(14)	-0.26754(9)	0.032(5)	***
C9	0.1121(10)	-0.3020(14)	-0.3534(9)	0.040(5)	***
C10	-0.0887(11)	-0.2879(17)	-0.3922(11)	0.055(6)	***
C11	0.1739(14)	-0.4704(15)	-0.2793(12)	0.064(7)	***

Table A.2. Fractional atomic co-ordinates for the hydrogen atoms of cylco-L-leucine-D-(allyl)glycine (13).

Atom	x	y	z
H21	0.4378	0.2896	-0.3440
H41	0.4610	-0.2540	-0.1748
H51	0.7279	0.4088	-0.1973
H52	0.7412	0.3263	-0.3570
H81	0.1441	-0.0250	-0.3282
H82	0.1565	-0.1416	-0.1743
H91	0.1518	-0.3033	-0.4464
H101	-0.1500	-0.4012	-0.4532
H102	-0.1333	0.1685	-0.4514
H103	-0.1252	-0.2835	-0.2978
H111	0.3162	-0.4796	-0.2538
H112	0.1130	-0.5796	-0.3457
H113	0.1374	-0.4751	-0.1849

Table A.3. Anisotropic thermal parameters for cylco-L-leucine-D-(allyl)glycine (*13*).

Atom	U11	U22	U33	U23	U13	U12
O1	0.045(4)	0.034(4)	0.032(4)	0.000(3)	0.017(3)	-0.003(3)
O2	0.054(4)	0.033(4)	0.031(4)	-0.002(3)	0.015(3)	-0.003(3)
N1	0.031(3)	0.030(4)	0.036(4)	0.002(4)	0.015(3)	-0.004(3)
N2	0.041(4)	0.027(4)	0.026(4)	0.005(4)	0.016(3)	-0.004(3)
C1	0.022(4)	0.034(5)	0.022(5)	0.004(4)	0.006(3)	0.005(4)
C2	0.035(4)	0.025(4)	0.031(4)	-0.004(4)	0.013(3)	-0.002(4)
C3	0.026(40	0.031(5)	0.022(5)	0.005(4)	0.005(3)	0.006(4)
C4	0.024(4)	0.024(4)	0.030(4)	0.003(4)	0.008(3)	-0.003(4)
C5	0.042(5)	0.044(5)	0.041(5)	-0.003(4)	0.016(4)	-0.014(4)
C6	0.036(5)	0.052(6)	0.069(7)	-0.013(6)	0.014(5)	0.000(5)
C7	0.056(6)	0.068(7)	0.067(7)	0.016(7)	0.015(5)	0.001(6)
C8	0.030(4)	0.032(5)	0.034(4)	0.002(4)	0.007(3)	0.000(4)
C9	0.042(5)	0.031(5)	0.045(5)	-0.009(5)	0.015(4)	-0.008(4)
C10	0.039(5)	0.055(6)	0.073(7)	-0.012(6)	0.004(4)	-0.010(5)
C11	0.063(7)	0.037(6)	0.091(9)	-0.010(6)	0.019(6)	-0.015)

Table A.4. Internulcear distances (Å) for cylco-L-leucine-D-(allyl)glycine.

O1...H1O2	2.91	1	-1.0	0.0	0.0
O1...N1	3.00	2	1.0	-1.0	-1.0
O1...H21	2.64	2	1.0	0.0	-1.0
O1...H52	2.98	2	1.0	0.0	-1.0
O1...H91	2.76	2	1.0	-1.0	-1.0
O2...N2	2.88	2	1.0	0.0	0.0
O2...H41	2.56	2	1.0	-1.0	0.0
O2...H51	2.79	2	1.0	0.0	0.0
O2...H111	2.72	2	1.0	-1,0	0.0
C1...H101	2.94	2	0.0	-1.0	-1.0
H21...C11	2.99	1	0.0	-1.0	0.0
C7...H113	2.99	1	-1.0	-1.0	0.0

Table A.5. Intramolecular distances (Å) for cylco-L-leucine-D-(allyl)glycine.

O1...N1	2.26	O1...C2	2.36
O1...H21	2.65	O1...C5	2.89
O1...H52	2.60	O2...N2	2.24

O2...C4	2.36	O2...H41	2.61
O1...C8	2.97	O2...H82	2.59
N1...N2	2.77	N1...C2	2.45
N1...C3	2.48	N1...H41	2.02
N1...C8	2.50	N1...H81	2.69
N1...H91	2.81	N2...C1	2.50
N2...H21	2.05	N2...C4	2.44
N2...C5	2.44	N2...H51	2.64
N2...C6	2.98	C1...H21	2.05
C1...C3	2.87	C1...C4	2.49
C1...C5	2.52	C1...H52	2.72
C2...C3	2.47	C2...C4	2.92
C2...H51	2.15	C2...H52	2.14
C2...C6	2.52	H21...C5	2.16
C3...H41	2.12	C3...C8	2.47
C3...H81	2.75	C3...H82	2.57
C4...H81	2.15	C4...H82	2.14
C4...C9	2.61	C4...H91	2.83
C4...H111	2.71	H41...C8	2.19
H41...C9	2.82	H41...C11	2.74
C5...C7	2.51	H51...C6	2.11

H51...C7	2.61	H52...C6	2.11
C8...H91	2.12	C8...C10	2.48
C...H102	2.71	C8...H103	2.69
C8...C11	2.49	C8...H111	2.72
C8...H113	2.74	H81...C9	2.12
H81...C10	2.65	H82...C9	2.1.
H82...C10	2.69	H82...C11	2.72
C9...H101	2.15	C9...H102	2.14
C9...H103	2.13	C9...H111	2.10
C9...H112	2.10	C9...H113	2.11
H91...C10	2.15	H91...C11	2.07
C10...C11	2.46	C10...H112	2.68
C10...H113	2.70	H101...C11	2.68
H103...C11	2.72		

APPENDIX TWO

Fig A1. ^{1}H NMR ($CDCl_3$) spectrum of t-butyloxycarbonyl-L-leucine-D,L-(allyl)glycine methyl ester.

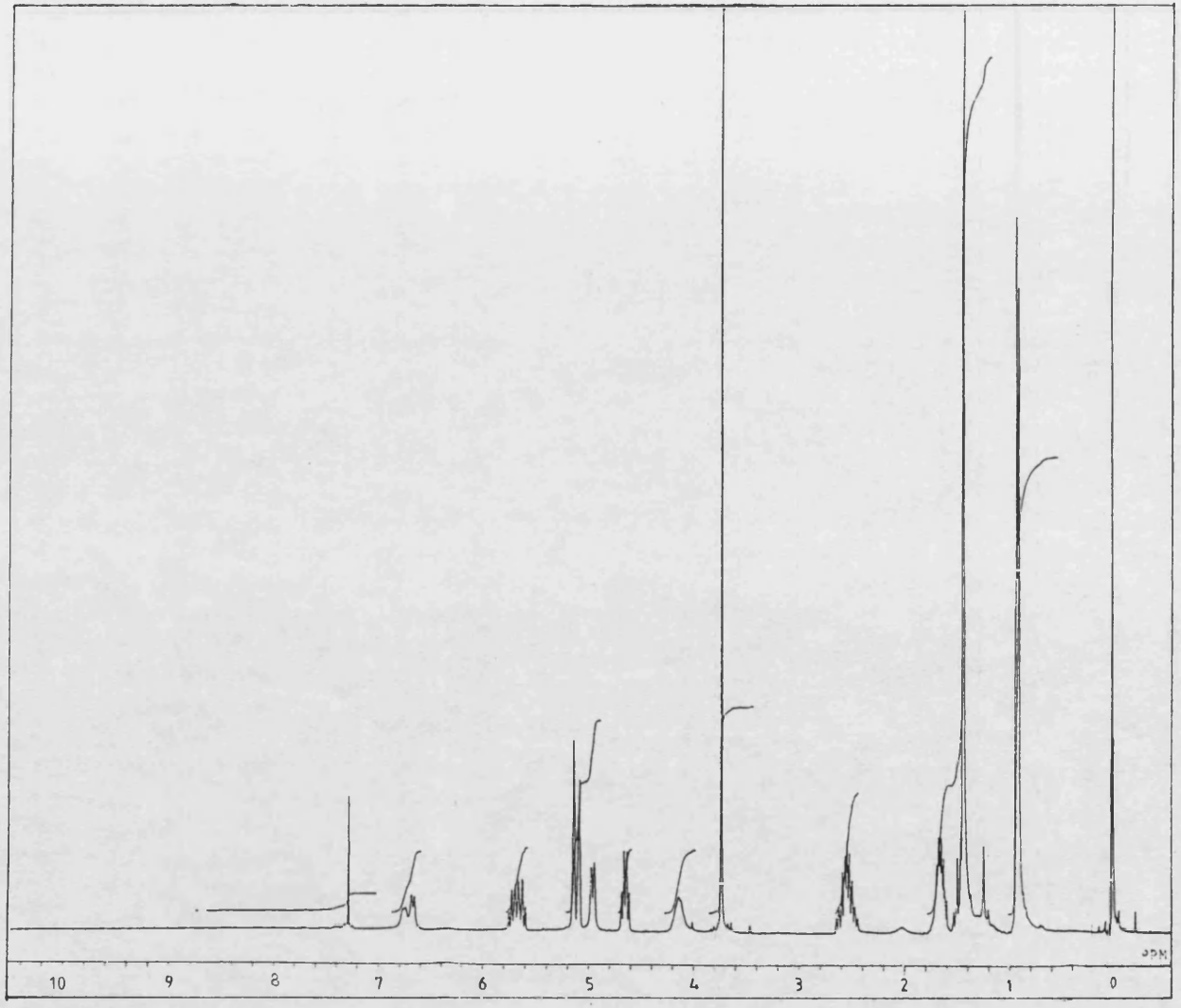


Fig A2. ^{1}H NMR (DMSO) spectrum of cyclo-L-leucine-D-(allyl)glycine.

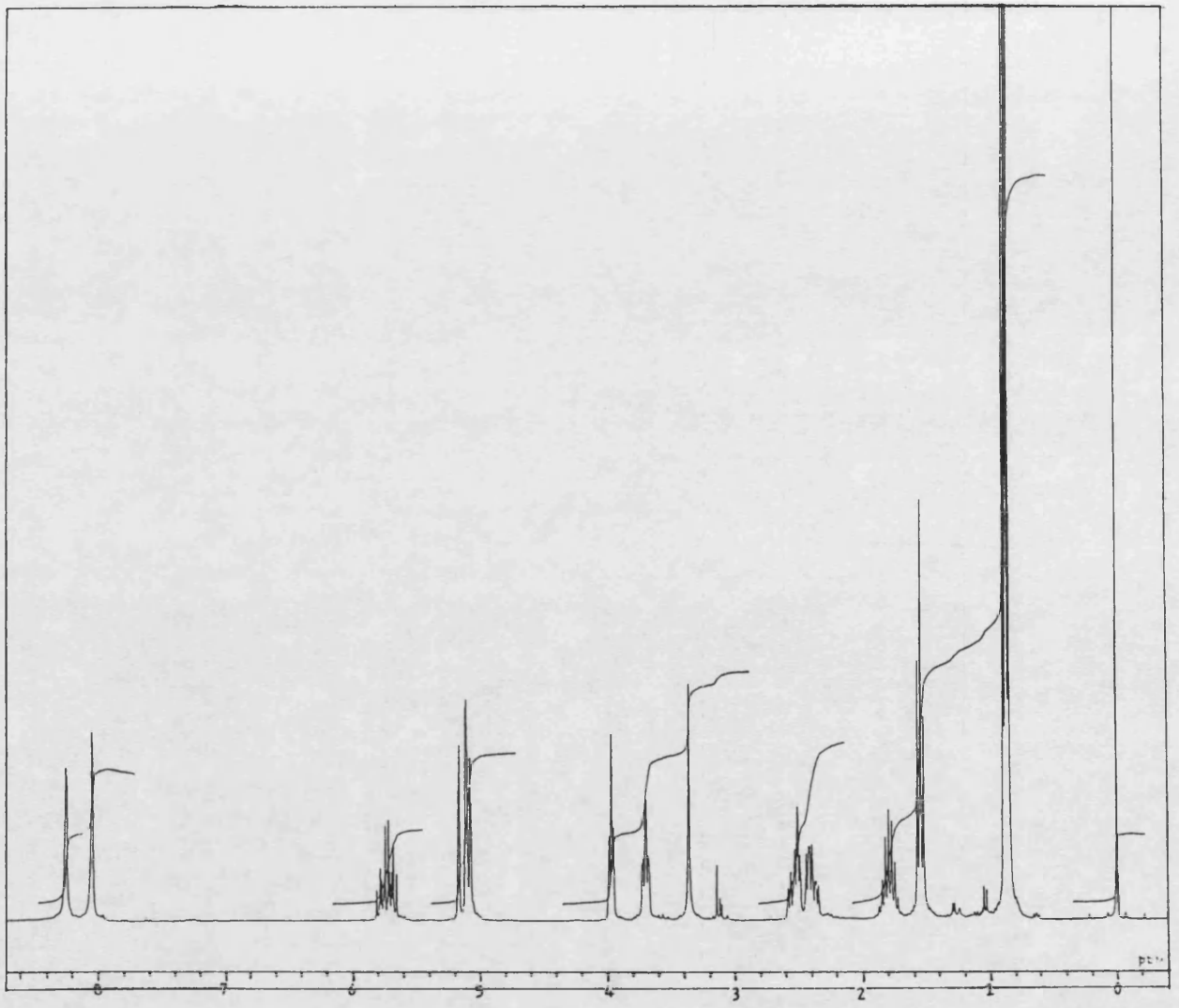


Fig A3. ^{1}H NMR ($CDCl_3$) spectrum of t-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester.

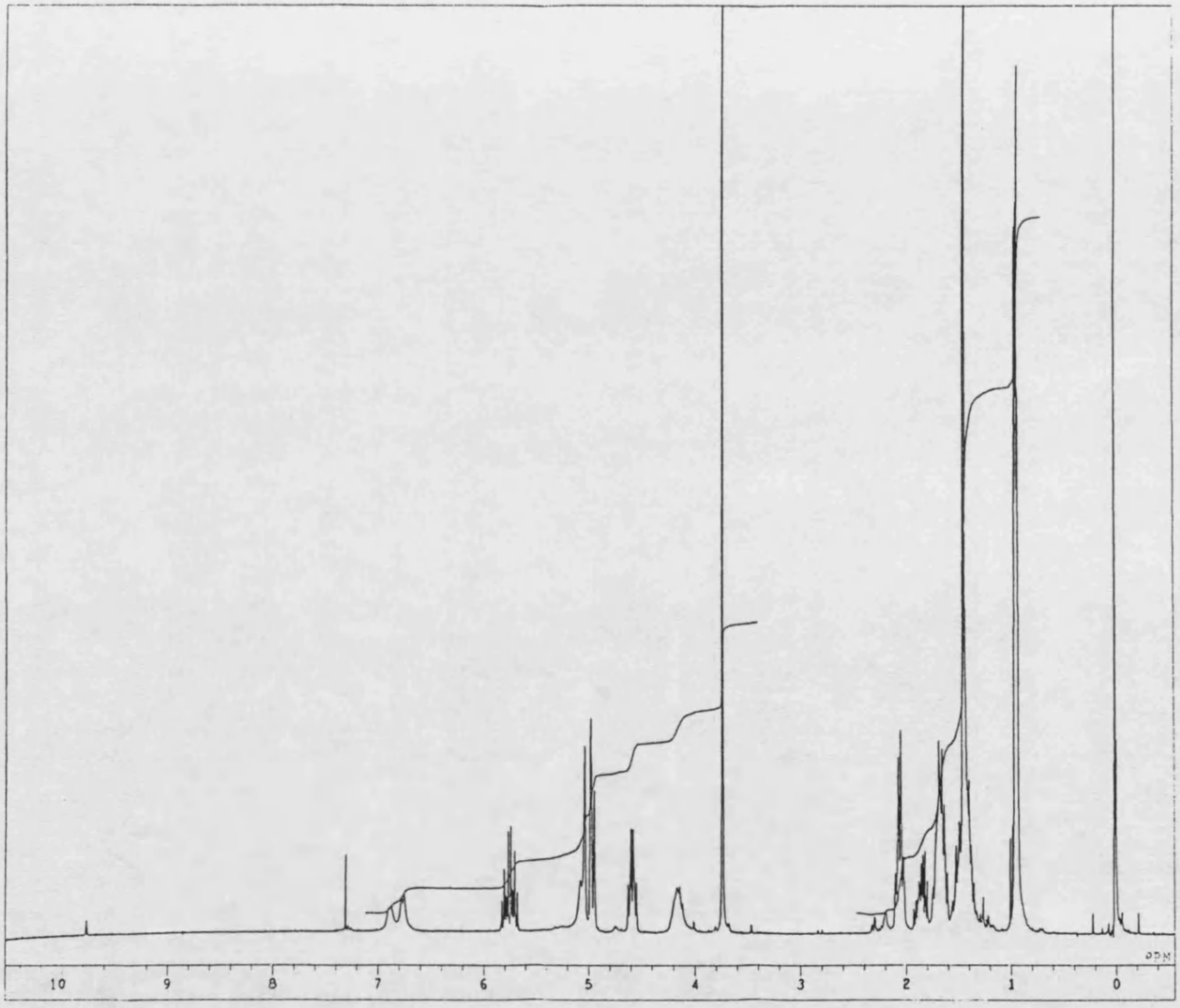


Fig A4. ^1H NMR ($CDCl_3$) spectrum of cyclo-L-leucine-D-(pentenyl)glycine.

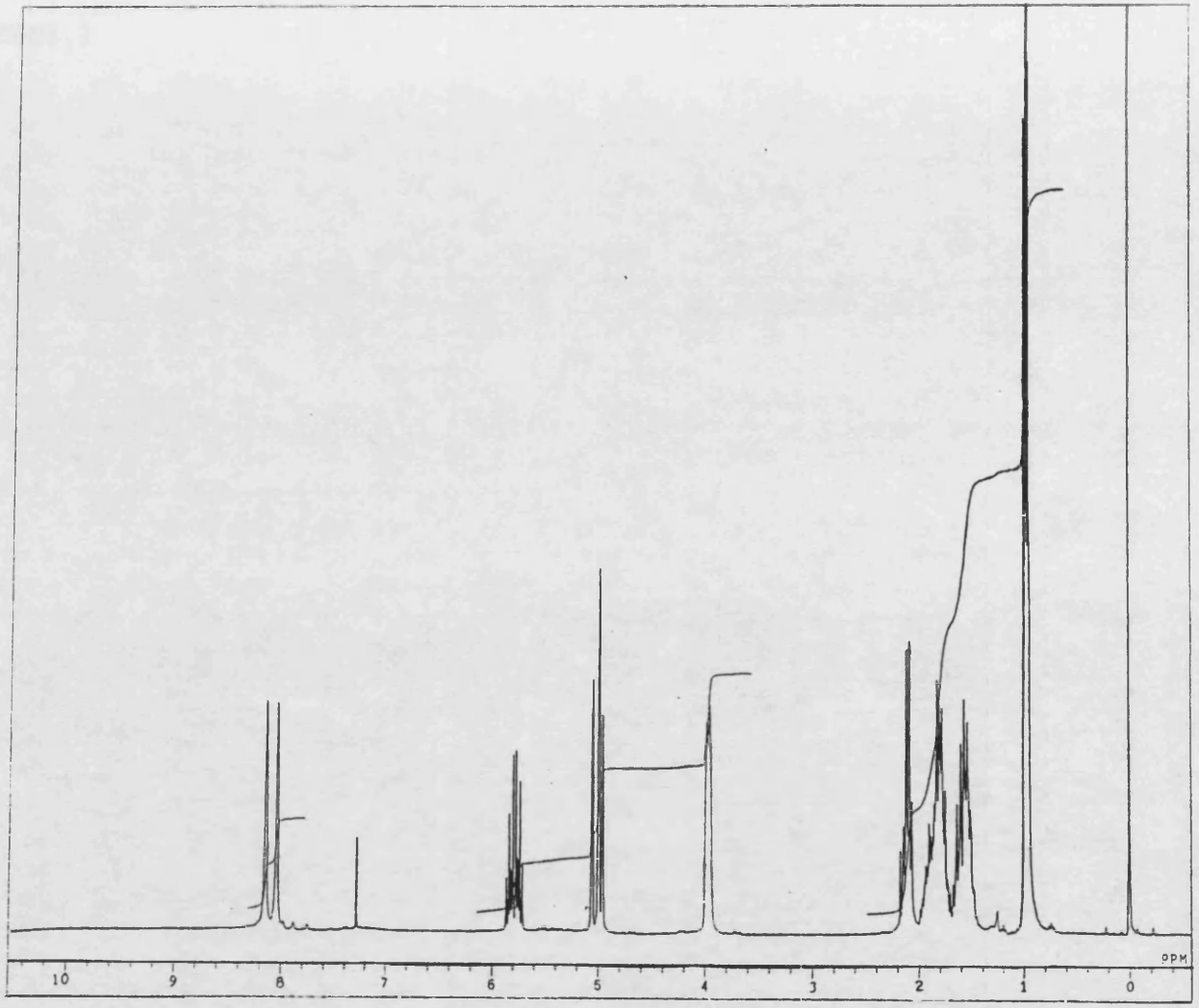


Fig A5. ^{1}H 2D COSY NMR ($CDCl_3$) spectrum of t-butyloxycarbonyl-L-leucine-D,L-(pentenyl)glycine methyl ester.

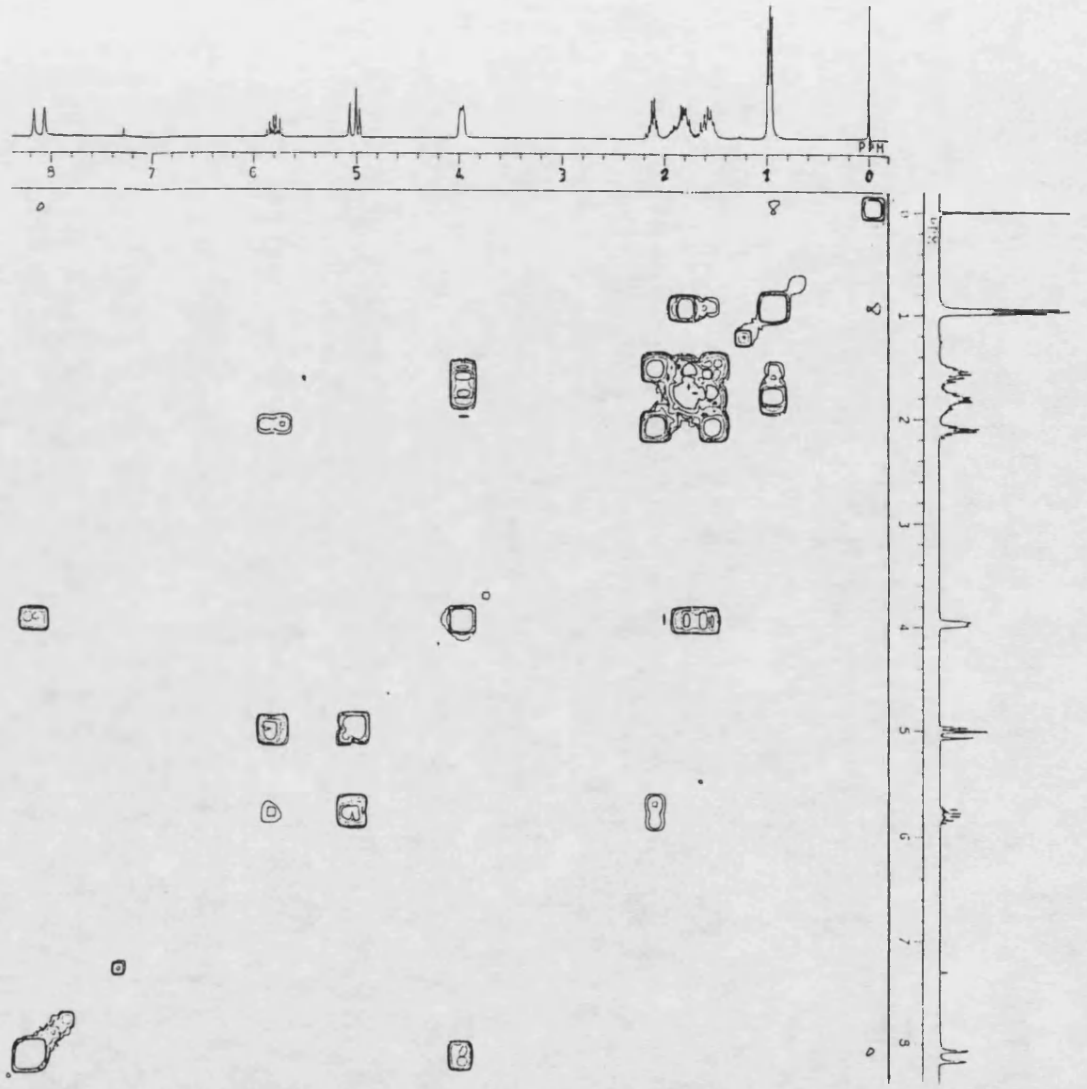


Fig A6. ^1H NMR ($CDCl_3$) spectrum of t-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucyl-D,L-(pentenyl)glycine methyl ester.

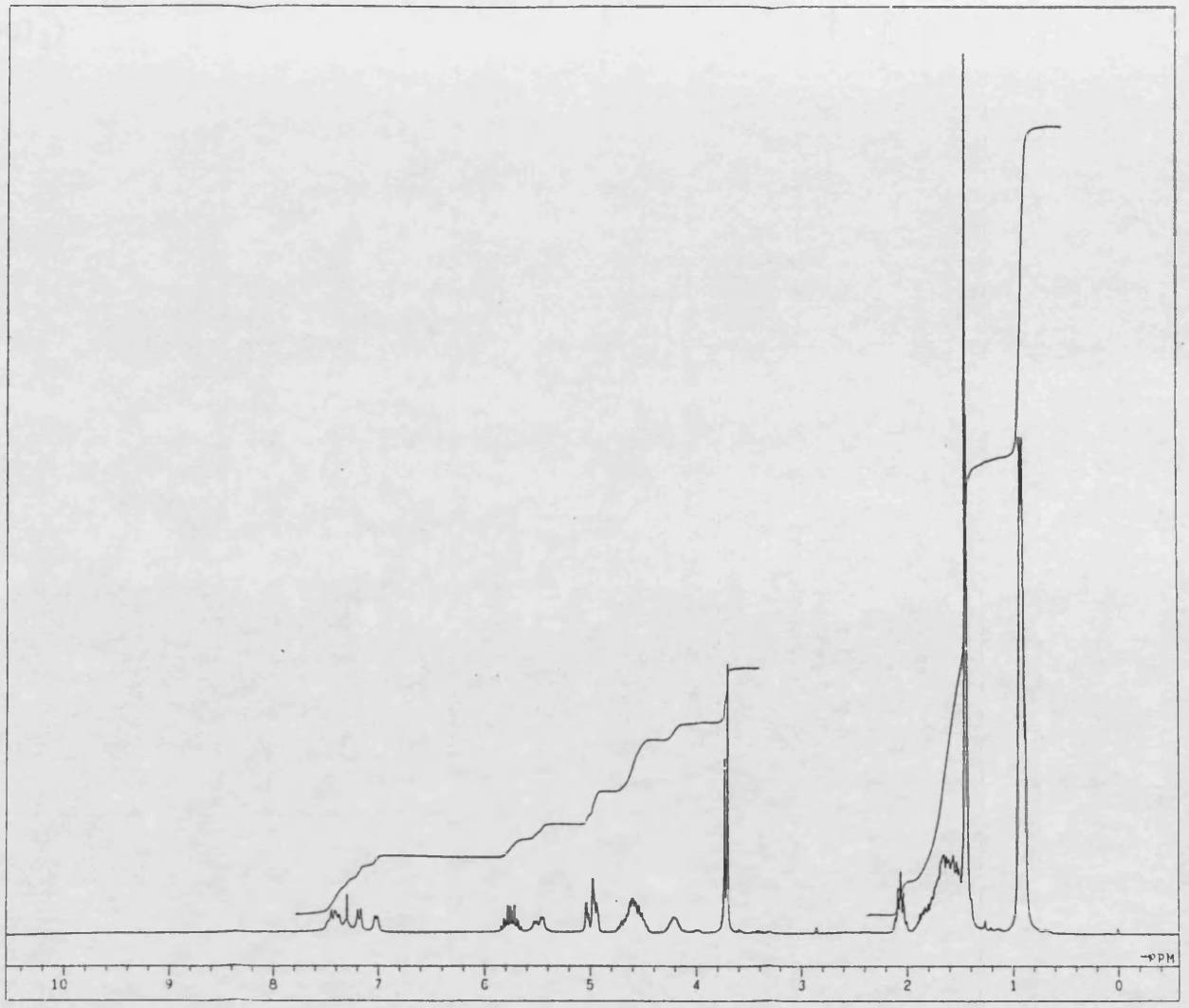


Fig A7. ^{1}H 2D COSY NMR ($CDCl_3$) spectrum of t-butyloxycarbonyl-L-leucyl-L-leucyl-L-leucyl-D,L-(pentenyl)glycine methyl ester.

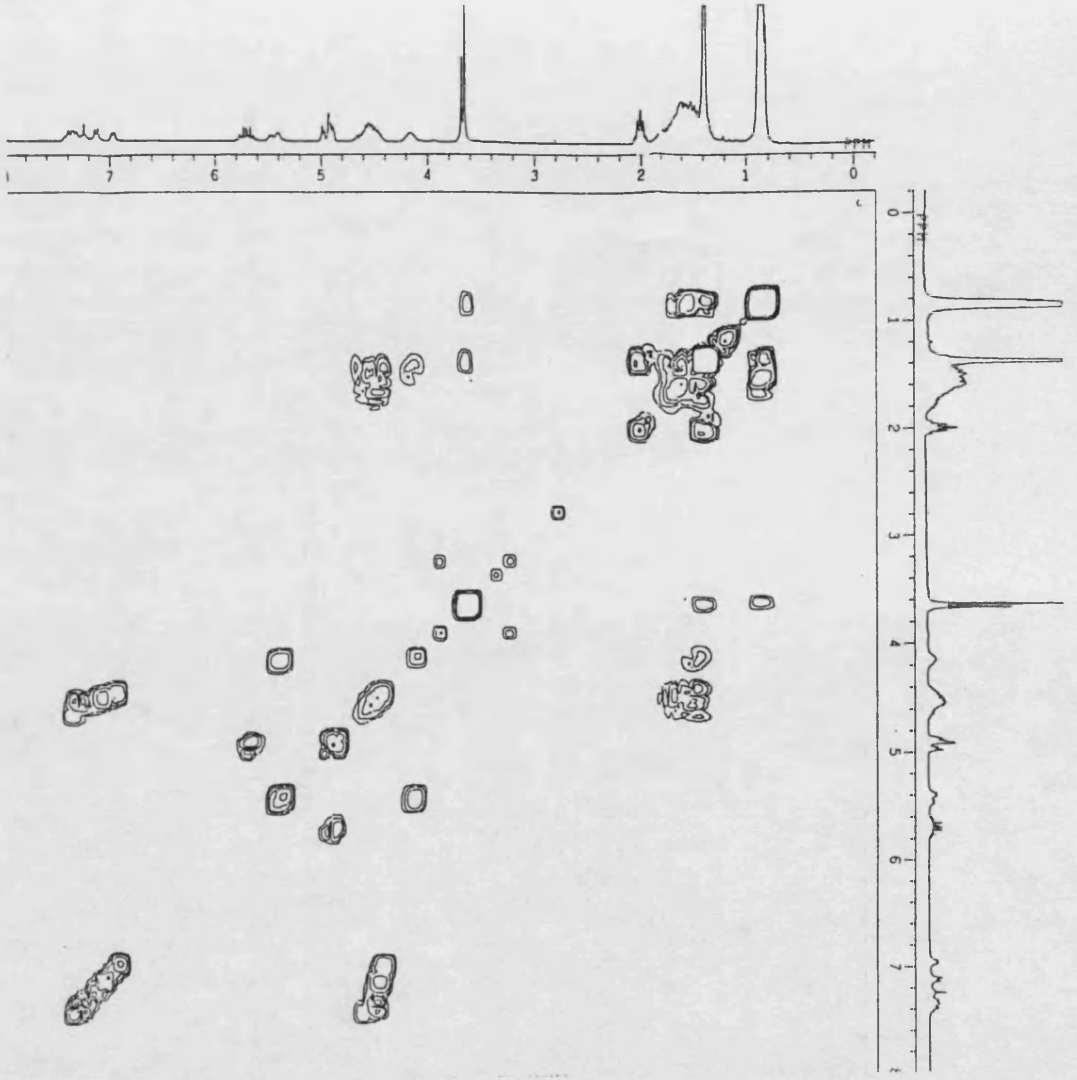


Fig A8. ^{1}H NMR (DMSO) spectrum of cyclo-L-leucine-D-(allyl)glycine triethyl silane.

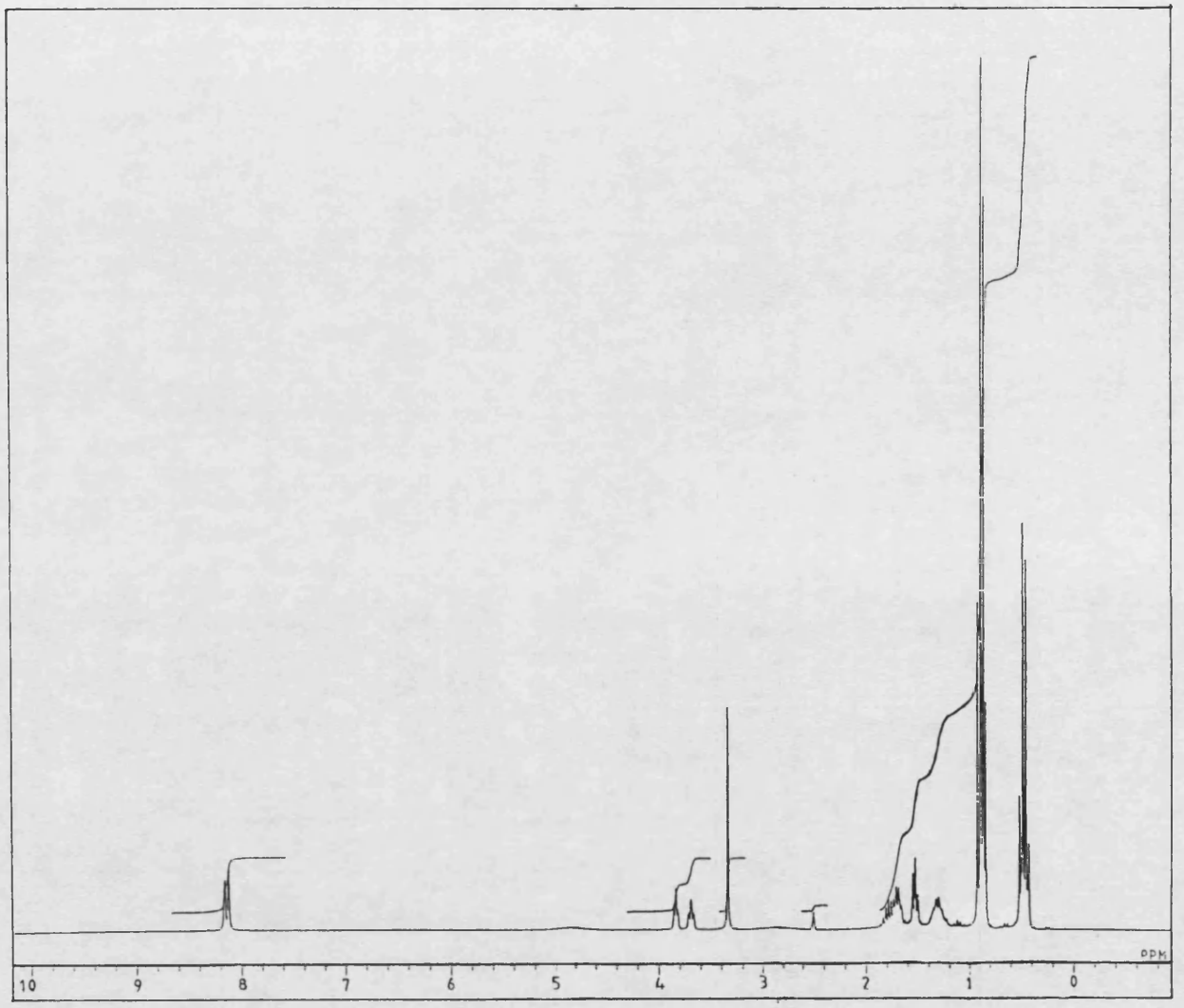


Fig A9. ^{1}H NMR (DMSO) spectrum of cyclo-L-leucine-D-(pentenyl)glycine triethyl silane.

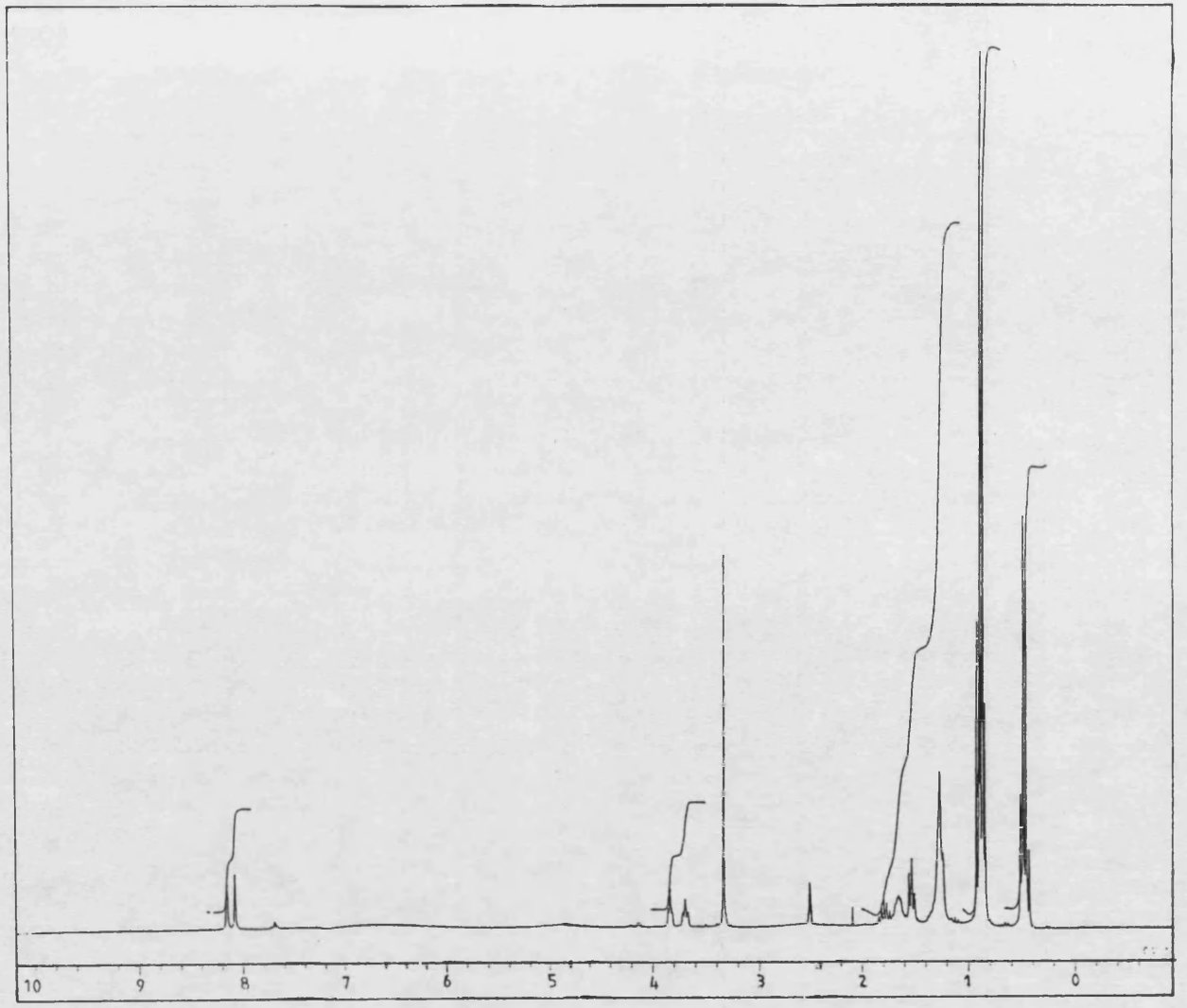


Fig A10. ^{1}H 2D COSY NMR (DMSO) spectrum of cyclo-L-leucine-D-(pentenyl)glycine.